Assessment of Toxicological Data for Sulfolane - Update II

AUGUST 18, 2010

ToxStrategies

Innovative solutions
Sound science

Assessment of Toxicological Data for Sulfolane – Update II

AUGUST 18, 2010

PREPARED FOR:
Flint Hills Resources
North Pole, Alaska Refinery

PREPARED BY:
ToxStrategies, Inc.
3420 Executive Center Drive
Suite 114
Austin, Texas
USA

Table of Contents

List of Abbreviations

ADEC	Alaska Department of Environmental Conservation
ATSDR	Agency for Toxic Substances and Disease Registry
BCMWLA	British Columbia Ministry of Water, Land, and Air Protection
BMD	benchmark dose
BMDL	95% lower confidence interval on BMD
BMDS	benchmark dose software
BMD_{1SD}	BMD with 1 standard deviation benchmark response
BMD_{1SDh}	BMD with 1 standard deviation benchmark response using historical data
BMR	benchmark response
CCME	Canadian Council of Ministers of the Environment
ECECB	European Commission, European Chemicals Bureau
GLP	good laboratory practices
HLS	Huntingdon Life Sciences
IUCLID	International Uniform Chemical Information Database
LD_{50}	lethal dose that results in death to half the treated animals
LOAEL	lowest observable adverse effect level
LOEL	lowest observable effect level
LUC	large unstained cells
NOAEL	no observable adverse effect level
NOEL	no observable effect level
OECD	Organisation for Economic Co-operation and Development
PDE	permitted daily exposure
SIDS	Screening Information Datasets
RfD	reference dose
RfC	reference concentration
RSL	Regional Screening Level
TDI	tolerable daily intake
UF	uncertainty factor
UF_A	interspecies uncertainty factor
UF_D	database uncertainty factor
UF_H	intraspecies uncertainty factor
UF_S	subchronic-to-chronic uncertainty factor
WBC	white blood cell

1.0 Introduction

ToxStrategies was retained by Flint Hills Resources to conduct an independent assessment of the toxicological data available for sulfolane. Our review addresses available background information, toxicity studies, screening levels in water, and our derivation of health protective toxicity factors and tap water screening levels for sulfolane. This version is an update to an earlier version where oral toxicity factors (reference doses) were based on the English translation of a Chinese article by Zhu et al. (1987). At the time of development of our prior assessment (Assessment of Toxicological Data for Sulfolane – Update, November 23, 2009), we did not have access to a proprietary GLP-conducted subchronic drinking water study performed by Huntingdon Life Sciences (HLS) (England) on behalf of Shell Canada. We have since obtained the report associated with the HLS study and have now considered, analyzed, and incorporated these data into our assessment of sulfolane. We have also located several additional sulfolane toxicity studies and have included discussions of these studies in this update as well.

1.1 Sulfolane – Background Information

1.1.1 Sulfolane Uses

Sulfolane is widely used as an industrial solvent. It is often used for gas treatment (for example, sour gas sweetening; hydrogen sulfide removal from shale and coal processes) and the removal of certain chemicals from waste streams. Sulfolane is also used in the manufacture of polymers and for various electronic applications. Sulfolane does not usually occur naturally in the environment. Worldwide production of sulfolane is 18,000 – 36,000 tons per year (Canadian Council of Ministers of the Environment [CCME], 2006b).

1.1.2 Fate and Transport

In the environment, sulfolane is poorly adsorbed to soil. Sulfolane has low volatility (essentially non-volatile) and high aqueous solubility. The chemical is mobile in the subsurface and is likely to be present in soil porewater. Sulfolane degrades slowly in groundwater relative to some other substances. In surface water, however, sulfolane degrades rapidly, (i.e., complete removal after 5 -11 weeks), particularly under aerobic conditions in the presence of sufficient nutrients (CCME, 2006b).

1.1.3 Taste & Odor

According to the International Uniform Chemical Information Database (IUCLID, European Commission, European Chemicals Bureau [ECECB], 2000) file, sulfolane concentrations below 580 ppb do not affect the smell or taste of water. (Note, however, the specific basis for this value of 580 ppb has not yet been confirmed.)

1.1.4 Routes of Exposure

Possible routes of human exposure to sulfolane in groundwater include ingestion of the chemical in drinking water, dermal contact with the chemical in tap water through bathing/showering and other activities, inhalation of the chemical as a mist while showering, and ingestion of the chemical in produce irrigated with sulfolane-contaminated groundwater. Another possible exposure route is through intrusion of the vapors through soils and into a home. However, of these potential exposure pathways, ingestion in drinking water, inhalation of the mist while showering, and ingestion of produce are the only potentially relevant exposure pathways. Sulfolane is considered nonvolatile (i.e., does not transfer easily from groundwater into the air), but it could be transferred into mists, which may be inhaled while showering. Sulfolane is not readily absorbed through the skin and has not been shown to be irritating or damaging to the skin surface or to the eyes, and thus is not a concern for bathing or other activities that could result in dermal contact (e.g., laundered clothes, dishwashing, etc). Flint Hills Resources is currently working with both the Alaska Department of Environmental Conservation (ADEC) and the Alaska Department of Health and Social Services (ADHSS) to assess the uptake of sulfolane in produce and, as such, this pathway will be addressed separately upon completion of the uptake studies.

1.1.5 Metabolism

Sulfolane is readily absorbed, distributed, and metabolized. Intraperitoneal (i.p.) administration of 100 mg/kg of ^{35}S-sulfolane was reported to result in the excretion of 85% of the radioactivity in the urine within the first 24 hours; with the major urinary metabolite identified as 3-hydroxysulfolane (Roberts and Warwick, 1961; Andersen et al., 1976). Intravenous administration of relatively high doses of sulfolane has been shown to result in the excretion of a large proportion of unchanged sulfolane (Andersen et al., 1976). Following i.v. administration of 500 mg/kg, about 36% of sulfolane was recovered within 4 days; whereas about 67% of a 1000 mg/kg dose was recovered within this timeframe – suggesting saturable metabolic pathway(s) (Andersen et al., 1976). Based on studies in rabbits, dogs and squirrel monkeys, the plasma half-life of sulfolane following i.v. administration was found to be about 3.5 to 5 hr (Andersen et al., 1976). Sulfolane has also been reported to have a large volume of distribution (Andersen et al, 1976)

The aforementioned results are in agreement with those published a decade later by Zhu et al. (1988), who examined the toxicokinetic aspects of ^{3}H-sulfolane following oral administration to Sprague-Dawley rats. In one series of experiments, the authors surgically opened rats along the abdomen and administered ^{3}H-sulfolane directly into sections of the gastrointestinal tract (stomach, jejunum or ileum), then ligated the segments and measured absorption after 10 hours. Although clearly not a physiologically relevant exposure scenario, they determined uptake in the jejunum and ileum to be nearly 80% within 4 hours, which was about twice the uptake from the stomach. In another set of experiments, rats were administered ^{3}H-sulfolane orally via gavage and the compound was subsequently found to distribute to the liver, kidney, lung, thyroid, pancreas, heart, adrenal gland, spleen, testis, muscle brain, and fat. Similar results were obtained in pregnant rats and sulfolane was also reported to be present in the placenta within two hours of exposure; however, the

gestational day of exposure was not stated. In the fetuses, sulfolane was detected in the liver, kidney, brain, lung, heart, muscle, and blood in descending concentrations. These data indicate that sulfolane can cross both the blood-brain barrier and the placenta.

2.0 Summary of Available Toxicity Data for Sulfolane (Hazard Identification)

There is a substantial body of data concerning sulfolane, including a host of genotoxicity studies, acute toxicity studies in multiple species via multiple routes of exposure, subchronic toxicity studies in multiple species via multiple routes of exposure, a chronic oral toxicity study, reproductive & developmental toxicity studies in multiple species via multiple routes of exposure, and a carcinogenicity study involving a structurally-related compound (sulfolene). In addition, two IUCLID files for sulfolane were obtained (ECECB, 2000; OECD, 2004); in some cases, these files describe the results of studies that are not publically available. The more recent IUCLID file (OECD, 2004) was peer-reviewed by the government of Japan. The following sections provide a summary of the available literature. A detailed listing of available toxicological studies is provided in **Table A1** in Appendix A. In Table A1, results from studies described in IUCLID files are cited as either ECECB (2000) or OECD (2004), followed by the citation number within those files.

2.1 Acute Toxicity

Brown et al. (1966) conducted several studies to investigate the acute toxicity of sulfolane. In these studies, the LD_{50} following a single oral gavage dose was determined to be 2,100 mg/kg in rats and ranged from 1,900 to 2,500 mg/kg in mice. Topical administration of 3,800 mg/kg sulfolane for 24 hours resulted in no overt toxicity. Sulfolane did not cause skin sensitization in guinea pigs, or eye irritation in rabbits. Undiluted sulfolane applied to the skin of rabbits and guinea pigs produced no gross signs of skin irritation and no histopathological signs of changes in skin (Brown et al., 1966).

Andersen et al. (1976) reported the single dose lethality of sulfolane in multiple species following multiple routes of exposure. In this study, oral LD_{50} values in rats and guinea pigs were determined to be 1,846 and 1,815 mg/kg, respectively. The LD_{50} values for rats and mice receiving sulfolane via intraperitoneal (i.p.) injection were determined to be 1,598 and 1,331 mg/kg, respectively. Intravenous (i.v.) administration to rats and mice resulted in LD_{50} values of 1,094 and 632 mg/kg, respectively; while subcutaneous (s.c.) injection resulted in LD_{50} values of 1,606 and 1,360 mg/kg, respectively (Andersen et al., 1976). By all routes, high doses of sulfolane induced signs indicative of CNS stimulation and toxicity (e.g. convulsions) in the Andersen et al. (1976) study. In another study, Zhu et al. (1987) reported oral LD_{50} values in mice, rats and guinea pigs of 2504, 2343 and 1445 mg/kg, respectively.

In a file obtained from the Organisation for Economic Co-operation and Development (OECD) Screening Information Datasets (SIDS) for High Volume Chemicals, we located a study conducted in accordance with good laboratory practices (GLP) and OECD Guideline 401 Acute Oral Toxicity Test (OECD, 2004). In this study, the oral LD_{50} values for male and female rats were determined to be 2,006 and 2,130 mg/kg, respectively. In another study included in IUCLID file (ECECB, 2000), sulfolane was reported to induce slight skin and eye

irritation. Another GLP OECD guideline study reported that sulfolane was negative for skin sensitization in the guinea pig maximization test (OECD, 2004).

Pharmacological studies indicated that sulfolane can lead to both CNS excitation and depression at very high does (approximately ½ LD_{50}). Sulfolane decreased barbiturate-induced sleeping time (CNS excitation) when given simultaneously with pentobarbital but also increased sleeping time (CNS depression) when given an hour before pentobarbital (Andersen et al., 1976). Similar CNS effects were observed following exposure to high concentrations of inhaled sulfolane. Andersen et al. (1977) reported that the highest achievable sulfolane air concentration in their exposure apparatus was 12,000 mg/m^3. Rats exposed to this concentration for 4 hours did not die within a two-week follow up period. Therefore, rats were continuously exposed to this concentration until all animals died; a plot of survival time resulted in an estimated acute (24 hr) 50% lethal concentration (LC_{50}) value of 4,700 mg/m^3. In a separate experiment, 9 rats exposed to 3600 mg/m^3 (i.e. 75% of the LC_{50}) for about 18 hours were found *in extremis*, were euthanized, and blood examined for hematological analysis (Andersen et al., 1977). A significant ($p < 0.05$, Student's t-test) decrease in white blood cell (WBC) count was observed relative to pre-exposure (4.2 ± 1.1 vs. 18.7 ± 0.5); however, hematocrit and hemoglobin levels did not differ significantly. Necropsy of all 9 rats revealed that all animals exhibited pulmonary hemorrhage. Two monkeys were exposed (via inhalation) to 4,850 mg/m^3 sulfolane and convulsed during exposure and were sacrificed after 19 hours. Both animals exhibited a) a more than a 25% reduction in WBC count, b) a more than 15% reduction in both hematocrit and hemoglobin, and c) pulmonary hemorrhage.

It is worth noting that several studies have examined the effects of acute exposure to high doses of sulfolane, many by i.p. administration, on thermoregulation in rodents (reviewed in ATSDR, 2010). However, the hypothermic effects of sulfolane appear to be reduced with increasing body mass (from mouse to rat to rabbit) (Gordon, 2005); thus the relevance to larger mammals (e.g. humans) is questionable. Although potentially of toxicological interest, the dose levels, routes and relevance of thermal effects for humans makes these studies less useful for risk assessment.

2.2 Repeated Dose Toxicity

Andersen et al. (1977) conducted a series of subchronic (≤ 90 days) inhalation toxicity studies with sulfolane in several species: squirrel monkeys, beagle dogs, rats, and guinea pigs. In one series of experiments, Andersen et al. exposed rats, guinea pigs, dogs, and monkeys to 495 mg/m^3 aerosolized sulfolane for 27 days. With the exception of monkeys, all animals survived the exposure and exhibited no signs of hematological or biochemical alterations. However, some rats exhibited signs of fatty liver, lung inflammation, and a slight but nonsignificant decrease in WBC count. Three monkeys died during exposure, and five were found *in extremis*. The monkeys exhibited signs of fatty liver and a slight but nonsignificant decrease in WBC count. In a second series of experiments, Andersen et al. exposed guinea pigs, dogs and two monkeys to 200 mg/m^3 for 23 hr/day for 90 days. The exposure was overtly toxic to both dogs and monkeys, and resulted in the deaths of both monkeys within 4 days (both monkeys exhibited parasitic infestations, and the authors

posited that this may have been a factor contributing to their susceptibility). This concentration led to convulsions and deaths in dogs. Unlike the earlier 27-day exposure carried out at 495 mg/m^3 for 8 hr/day for 5 days per week, guinea pigs continuously exposed to 200 mg/m^3 sulfolane (23 hr/day for 90 days) exhibited a significant reduction in WBC at 20, 30 and 90-days after the start of exposure as well as fatty liver at study termination. Although statistically significant, the biological significance is unclear. In fact, the mean values before exposure, after 20, 30, 60 and 90 days were 5.9, 3.1, 3.8, 5.2 and 4.4, respectively. These values indicate that the decrease did not necessarily progress with time. However, in a third experiment, guinea pigs exposed to a slightly lower concentration, 159 mg/m^3 23hr/day for 85 days, exhibited no statistically significant changes in WBC when examined 30, 60 and 85 days after start of the exposure. In another series of experiments, Andersen et al. reported that continuous exposure to 20 mg/m^3 sulfolane for 23hr/day for 90 days resulted in no overt signs of toxicity in rats, guinea pigs, dogs and monkeys. Andersen and colleagues thus considered 20 mg/m^3 a "no-effect level." A potential caveat to the findings regarding WBC count is that the statistical results were not provided separately for male and female guinea pigs. These values can differ between sexes. Nevertheless, the findings suggest a threshold for effects on WBC count between 159 and 200 mg/m^3.

Zhu and co-workers (1987) conducted several toxicity studies on sulfolane, including 90-day studies in rats and guinea pigs, and a chronic (6-month) oral toxicity study in newly-weaned guinea pigs. In the 90-day study, animals were exposed to 55.6, 167, and 500 mg/kg sulfolane. Changes in serum chemistry were observed in rats exposed to 500 mg/kg/day, whereas serum chemistry and hematological changes (decreased WBC) were observed in guinea pigs starting at 55.6 mg/kg/day. Specifically, serum alkaline phosphatase (ALP) was reduced in the two lower groups and presumably unchanged in the high dose group. The total WBC count was reported to decline in all the dose groups in guinea pigs. Although Zhu et al. considered these changes significant, the statistical tests are not described, and more importantly, mean and standard deviation data were not provided. It is also noteworthy that data for changes in WBC counts were not reported separately for males and females. In fact, WBC counts differ between males and females.

Due to the apparent sensitivity of guinea pigs, Zhu et al. (1987) performed a chronic oral toxicity study in guinea pigs. In the 6 month study, guinea pigs were administered sulfolane at doses of 0.25, 2.5, 25, and 250 (mg/kg/day). After only 3 months of exposure, some animals were sacrificed and toxicological endpoints were measured. Again, changes in serum enzyme levels were reported without measures of variability. Similarly, reductions in bone marrow cell counts were said to be reduced but there were no measures of variability. The serum enzymes reported to be decreased were aspartate aminotransferase (AST) and alanine aminotransferase (ALT); these enzymes were formerly called glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) – the latter terminology was used in Zhu et al. (1987). Zhu and colleagues did not provide any indication of normal reference ranges for these enzymes, thus the biological significance of these findings are unclear. Zhu et al. (1987) also characterized the serum enzyme levels and bone marrow counts as "unchanged" from controls in the lowest treatment group. It seems highly unlikely that the values for these 3 endpoints would be identical between any

two groups. Without measures of variability it is nearly impossible to interpret the significance of these findings. Zhu et al. (1987) also provided incidence data for dispersion of the white pulp of the spleen at 90 days; the incidences were 0/14, 0/14, 1/14, 2/14, and 6/14 for the control and four treatment groups, respectively. After 6 months of exposure, Zhu et al. (1987) reported incidence data for fatty degeneration of the liver, heavy fatty degeneration of the liver, and dispersion of the white pulp of the spleen. The incidence for fatty degeneration of the liver in the control and treated animals were 0/25, 0/22, 2/26, 4/25, 7/22. The incidence for heavy fatty degeneration of the liver in the control and treated animals were 0/25, 0/22, 1/26, 2/25, 5/22. The incidence for dispersion of the white pulp of the spleen in the control and treated animals were 0/25, 0/22, 2/26, 2/25, 7/22. Based on the various findings observed in their studies, Zhu et al. identified no effect level of 0.25 mg/kg. This interpretation of the NOAEL will be discussed in greater detail in Section 3.

Huntingdon Life Sciences (HLS, 2001) conducted a GLP certified subchronic (90-day) drinking water study for sulfolane in CD (Sprague Dawley) rats. In this study, male and female rats were exposed to concentrations of 0, 25, 100, 400, or 1,600 mg/L sulfolane (2.1, 8.8, 35, and 132 mg/kg/day in male rats and 2.9, 10.6, 42 and 191 mg/kg/day in female rats) *ad libitum*. Ten male and 10 female rats were exposed to each drinking water concentration. For quality assurance, samples of each sulfolane formulation prepared for administration were analyzed for achieved concentration at weeks 1, 6 and 12 of the study. The animals were thoroughly examined for signs of adverse health effects. Examinations included: food and water consumption, bodyweight, organ weights, functional observations (e.g. reflexes, grooming, motor activity), hematological evaluations, blood chemistry, gross pathology, and histopathological examination of 13 of the major organs (adrenals, brain, femur, heart, ileum, kidneys, liver, lungs, mammary area, spinal cord, stomach, thyroid, and uterus).

The exposure was described as well tolerated, and the study authors identified two primary effects of concern following oral exposure to sulfolane. Male rats exhibited treatment related effect in kidneys involving both cortical cell basophilia and hyaline droplets. This form of renal toxicity in male rats is well documented and involves chemical-induced inhibition of alpha-2u-globulin catabolism leading to accumulation in secondary lysosomes that appear transparent (hyaline-like) via microscopy. This protein appears to be specific to male rats and thus the hyaline droplets are not considered to be relevant to humans (U.S. EPA, 1991; Hard et al., 1993; HLS, 2001). In fact, USEPA has concluded that alpha-2u-globulin hyaline droplet formation is unique to male rats and is probably not relevant to humans for purposes of risk assessment (U.S. EPA, 1991). The basophilia observed likely relates to cell proliferation (tubular regeneration) subsequent to alpha-2u-globulin accumulation. Indeed the HLS study authors attributed this and the presence of granular casts (cell debris) in the highest male dose group to “hydrocarbon nephropathy” due to alpha-2u-globulin accumulation. According to Hard et al. (1993), “granular casts stain positive for alpha-2u-globulin, indicating probable derivation of debris from cells that had accumulated this protein.” Based on the aforementioned considerations, these endpoints were not considered relevant for the assessment of sulfolane.

The other effect considered to be treatment-related by the HLS study authors was a decrease in lymphocyte, monocyte and large unstained cell counts (and a concomitant decrease in total white blood cell (WBC), or leukocyte, counts) in female rats administered 100, 400, or 1600 mg/l (10.6, 42 and 191 mg/kg/day, respectively), though the study authors concluded that these effects did not follow a strong trend with dose. Additionally, the study authors noted that there was no evidence of any chronic inflammatory change or compromised immune function in females, nor were there any effects on bone marrow, thymus or spleen that might account for reduced numbers of white blood cells. As such, the study authors concluded that the toxicological significance of the effects on white blood cells was unclear. Further complicating the understanding of the toxicological significance of these findings is the fact that these effects were not observed in male rats.

The IUCLID file (ECECB, 2000) includes a study that found no adverse effects on bodyweight, behavior or blood (details not provided) in rats (unspecified sex) exposed orally to 50 mg/kg sulfolane 6 times per week for 4 months. Another IUCLID file (OECD, 2004) contains a subchronic GLP study in rats exposed orally at doses of 60, 200, and 700 mg/kg/day. In this study, male and female rats were exposed daily by oral gavage for 28 days, with a 14 day post-exposure observation period. Clinical observations were performed and bodyweight and food consumption were determined. Hematological and blood chemical analysis were carried, as were urinary analyses. Organs were weighed and examined at the time of necropsy included: brain, heart, liver, kidneys, spleen, thymus, adrenal glands, testes, epididymides, and ovary. Microscopic examinations included kidney, brain, spinal cord, pituitary, eye ball, thyroid, thymus, heart, trachea, lung, liver kidneys, spleen, adrenals, stomach, small intestines, pancreas, testes, epididymides, prostate, ovary, uterus, vagina, bladder, lymph node, sciatic nerve, bone marrow. The study authors reported a NOAEL of 200 mg/kg/day in females based on reduced bodyweight gain and food consumption, and slight increase in blood enzymes. In males, the NOAEL was determined to be 60 mg/kg/day based on histopathological changes in the kidney. As noted above, this effect likely involves alpha-2u-globulin accumulation that is unique to the male rat and, as such, is not relevant for human risk assessment.

2.3 Reproductive and Developmental Effects

Data regarding potential reproductive and developmental effects were identified in three data sources. IUCLID file (OECD, 2004) includes data regarding a subchronic reproductive GLP study in rats (an OECD Guideline Study 421 "Reproductive/Developmental Toxicity Screening Test.") In this study, male and female rats were exposed daily by oral gavage to 60, 200, and 700 mg/kg/day for 41-50 days. This study identified a NOAEL of 700 mg/kg/day for males based on reproductive performance, and a NOAEL of 200 mg/kg/day for females based on a reduction in the number of estrus cycles in the 700 mg/kg/day group (OECD, 2004). As a part of this study, female rats continued to receive sulfolane throughout gestation and over the first three days of lactation. The pups were subject to clinical and full macroscopic evaluations once a day. At 200 mg/kg/day, birth index (live pups born divided by number of implantation sites ×100) and the number of pups alive at day 0 and day 4 of lactation were decreased. Pup bodyweight was also reduced significantly ($p < 0.01$) at 700 mg/kg. Based on birth index and live pups at day 0 and day 4, the IUCLID file reported a NOAEL of 60 mg/kg/day for the pups. According to OECD (2004),

there were "no treatment-related findings in the external appearance, general conditions and necropsy findings of the offspring."

The OECD Guideline 421 study states that this test does not provide complete information on all aspects of reproduction and development, but offers a limited means of detecting post-natal manifestations of prenatal exposure, or effects that may be induced during post-natal exposure. However, the guideline further states that "[a]lthough…negative data do not indicate absolute safety with respect to reproduction and development, this information may provide some reassurance if actual exposures were clearly less than the dose related to the No-Observed-Adverse Effect Level (NOAEL)."

In Zhu et al. (1987), Kunming mice were exposed to 93, 280, 840 mg/kg sulfolane on gestation days 6-15. On the 18th day of gestation, the dams were sacrificed and the fetal mice were examined. Some abnormalities were observed at the highest dose, and fetal resorptions were also increased at the highest dose. No abnormalities or resorptions were observed in the 280 mg/kg dose group. In addition to the study by Zhu et al (1987), a secondary source (ICH, 2005) provided a summary of an unpublished Glaxo Wellcome study, where pregnant Sprague-Dawley rats were exposed to 25, 100, and 400 mg/kg sulfolane (via subcutaneous administration) from gestation days 6-15. No embryolethal or teratogenic effects were observed, however fetal weight was marginally reduced at the high dose. The no-observed effect level (NOEL) for teratogenicity in this study was identified as 400 mg/kg.

2.4 Genotoxicity and Carcinogenicity

Several in vitro studies were negative for sulfolane-induced mutagenicity (**Table A2** in Appendix A). IUCLID file (ECECB, 2000) describes a GLP-conducted study that reported negative results for sulfolane mutagenicity in the Ames test with five Salmonella typhimurium strains (TA1535, TA1537, TA1538, TA98, and TA100) with and without metabolic activation. The same study reported negative results in an Escherichia coli reverse mutation assay with and without activation in WP2 and WP2 uvrA strains. Negative results were also found in Saccharomyces cerevisiae with and without activation. Studies in mammalian RL4 cells were negative without activation. In a separate study reported in ECECB (2000), sulfolane was negative for genotoxicity in Chinese hamster V79 cells (no data on activation was provided).

In a more recent IUCLID file (OECD, 2004), a different GLP study, conducted according to OECD Guidelines 471 and 472, reported negative results for sulfolane mutagenicity in the Ames test with four Salmonella typhimurium strains (TA1535, TA1537, TA98, and TA100) with and without activation. Negative results were also found in a chromosomal aberration test in mammalian Chinese hamster CHL/IU cells with and without metabolic activation. Negative results for sister chromatid exchange were reported in a non-GLP study in Chinese hamster Ovary cells with and without activation. A non-GLP study also reported negative results in TA1535, TA1537, TA1538, TA90, and TA100 (different from presented above). This IUCLID file also reported negative results for some of the studies included in ECECB (2000). A single study has reported positive results for genotoxicity. According to

OECD (2004), a non-GLP mouse lymphoma assay was positive for forward mutations (with and without activation). Although the study authors concluded that the results were positive, the IUCLID file summary section considered the interpretation to be "incorrect" due to the obvious lack of dose response (OECD, 2004). The IUCLID file Mutagenicity section concluded that for sulfolane there were no in vivo[1] mutation studies, and that sulfolane was not mutagenic to bacteria or mammalian cells in vitro.

Zhu et al. (1987) conducted three separate studies on sulfolane genotoxicity. Sulfolane was negative in five strains of Salmonella typhimurium with and without activation. Sulfolane did not significantly increase sister chromatid exchange in human peripheral lymphocytes treated in vitro at 0.01, 0.1, 1 and 10 mg/mL – although the highest dose was cytotoxic. In the third study, 7-week old mice were administered oral doses of sulfolane at 62.5, 125, 250, 500, 1000 mg/kg (the number of animals was not specified). Zhu et al. stated that micronucleus rate in each dose group did not differ from the negative control (water only).

There are no reported cancer studies for sulfolane; however, a structurally similar compound (sulfolene) was found to not cause any exposure-related neoplasms in rats or mice exposed via gavage in a study conducted by the National Cancer Institute (NCI, 1978). The NCI study report also indicated that two additional structurally similar compounds (3,4-epoxysulfolane and 1-propanesulfonic acid-3-hydroxy-gamma-sultone) were found to induce a variety of benign and malignant tumors under similar testing conditions. However, sulfolene is more similar structurally to sulfolane than are these two additional compounds (see Appendix A for structures).

Taken together, the lack of evidence of genotoxicity for sulfolane and the negative cancer bioassay results for a structurally similar compound indicate that there is currently no basis for deriving reference values based on cancer.

2.5 Potential for Asthma Exacerbation

No evidence was located indicating any association between sulfolane (or its metabolites) and asthma or decreased pulmonary function. As noted above in Section 2.1, Andersen et al. (1977) examined the effects of inhaled sulfolane in several species. Chronic pulmonary inflammation occurred in exposed animals at ≥ 200 mg/m^3, but persistent, substantial changes in breathing patterns were not observed at these dose levels (≥ 200 mg/m^3). During intermittent times of convulsive activity, the animals were observed to have deep, slow, labored breathing. However, the authors concluded this effect could result from either pulmonary or CNS toxicity, or be due to a combination of the two. Exposure to ≤ 20 mg/m^3 for 90 days did not induce any overt effects. Together with the evidence indicating that sulfolane is not a dermal irritant or sensitizer, available information suggests that sulfolane is not likely to exacerbate asthma. Nonetheless, at very high concentrations in air, sulfolane might cause pulmonary irritation or damage that could in turn exacerbate asthma. Importantly, sulfolane is considered a non-volatile compound and, as such, the possibility for inhalation exposure is greatly reduced.

[1] This IUCLID file did not include Zhu et al. (1987).

3.0 Derivation of Toxicity Factors (Dose Response Assessment)

Review of a number of common sources of available toxicity factors including U.S. EPA's Integrated Risk Information System, U.S. EPA's Provisional Peer-Reviewed Toxicity Values, U.S. EPA's National Center for Environmental Assessment provisional values, the State of California's toxicity databases, and the Agency for Toxic Substances and Disease Registry's Minimal Risk Level list indicated that toxicity factors were not available from any of these sources. Additionally, sulfolane is not included in U.S. EPA's Regional Screening Level (RSL) tables (U.S. EPA, 2009b). As such, ToxStrategies reviewed available primary toxicology literature, summary documents, and reviews to determine appropriate oral and inhalation toxicity factors for sulfolane. As described above in Section 2, there was some evidence of noncarcinogenic effects in animals exposed to sulfolane. However, the lack of evidence of genotoxicity and negative cancer bioassay results for a structurally-similar compound indicate that there is currently no basis for deriving toxicity factors based on cancer. As such, the focus in this section is on development of toxicity factors based noncarcinogenic effects.

The primary toxicity factors of interest for noncarcinogenic effects are the oral reference dose (RfD) and the inhalation reference concentration (RfC). These toxicity factors are defined by U.S. EPA's Integrated Risk Information System (IRIS, U.S. EPA, 2009a), as follows:

> ***Reference Dose (RfD):*** *An estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. It can be derived from a NOAEL, LOAEL, or benchmark dose, with uncertainty factors generally applied to reflect limitations of the data used. Generally used in EPA's noncancer health assessments. [Durations include acute, short-term, subchronic, and chronic and are defined individually in the IRIS glossary].*
>
> ***Reference Concentration (RfC):*** *An estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. It can be derived from a NOAEL, LOAEL, or benchmark concentration, with uncertainty factors generally applied to reflect limitations of the data used. Generally used in EPA's noncancer health assessments. [Durations include acute, short-term, subchronic, and chronic and are defined individually in the IRIS glossary].*

3.1 Derivation of Toxicity Factors by ToxStrategies

Historically, traditional approaches to risk assessment employ relatively simple methods, sometimes called default approaches, where lowest observable adverse effect level (LOAEL) or no observable adverse effect level (NOAEL) values are divided by various uncertainty factors (UFs) to develop an RfD or RfC (U.S. EPA, 2002a). Limitations of LOAEL/NOAEL approaches include: a) the LOAEL/NOAEL is limited to the doses tested, b) the LOAEL/NOAEL does not appropriately reflect study size, c) the LOAEL/NOAEL cannot be directly compared across studies and endpoints based on a common response level (e.g. 10% increased risk), and d) the approach can inappropriately reward poorer studies with less statistical power to detect effects resulting in higher LOAEL and NOAEL values. As a result, over time, USEPA and others have been moving towards development of more sophisticated approaches involving modeling. In fact, one such tool, termed benchmark dose (BMD) modeling has been recognized as the preferred alternative because it takes into account the shape of the dose-response curve, the confidence limits reflect the size of the study, and allows comparison of comparable results across studies and endpoints at any response level (e.g. 10% increased risk) (Allen et al., 1998; Crump, 1984; Gaylor et al., 1998; Leisenring and Ryan, 1992; U.S.EPA, 2000, 2002a). Where possible, ToxStrategies has attempted to employ BMD modeling for the evaluation of sulfolane.

3.1.1 Oral Exposure Route

Examination of the available toxicity data for studies with information potentially useful for dose-response modeling indicated that the three most relevant oral studies were a repeated dose study originally published in Chinese by Zhu et al. (1987), a proprietary unpublished subchronic GLP study conducted by HLS (2001), and a GLP OECD Guideline Study summarized in an IUCLID file (OECD, 2004). After obtaining a full English translation of Zhu et al. (1987), we were able to determine that Zhu et al. (1987) contained incidence data for multiple endpoints that could be amenable to dose-response modeling in accordance with U.S. EPA's preferred approach of developing data-derived toxicity benchmarks. We were also able to obtain a copy of the HLS (2001) study, which also contained data amenable to dose-response modeling. Thus as an alternative to simply working with the unconfirmed NOAEL values reported by CCME in their water quality guidelines for sulfolane (CCME, 2006a), we qualitatively and quantitatively analyzed the data reported in Zhu et al. (1987) and HLS (2001). We also modeled developmental toxicity data summarized in an IUCLID file (OECD, 2004); and moreover, the data were checked against the original Japanese report that had data tables written in English. The dose-response modeling of these studies are described in more detail below.

3.1.1.1 HLS (2001)

The HLS study investigators reported statistically significant (based on Williams' Test) decreases in WBC, lymphocyte, monocyte, and large unstained cell counts in female rats given 100 mg/l (10.6 mg/kg/day) or more sulfolane. However, as already noted above in Section 2.2, even though these decreases were statistically significant relative to the concurrent control animals, the HLS study investigators concluded that the toxicological significance of the effects on WBC counts was unclear due to the lack of evidence of any

chronic inflammatory change or compromised immune function in female rats, as well as lack of any effects on bone marrow, thymus or spleen that might account for reduced numbers of white blood cells.

To more closely examine these hematological effects, we performed a series of statistical analyses for total WBC counts, as well as on counts of the various WBC components – including lymphocytes, basophils, monocytes, and large unstained cells (LUCs). As is evident in **Table 1**, pair wise t-tests on the means of each dose group relative to controls indicated a statistically significant decrease (p-value ≤ 0.05) at the three highest dose levels tested (100, 400, and 1600 mg/L or 10.6, 42 and 191 mg/kg/day). In addition, trend tests (1- and 2-sided Joncheere's test; Jonckheere, 1954) demonstrated a statistically significant decreasing trend for total WBC counts, as well as counts of all four WBC components, as dose increased.

Table 1

Statistical Analyses of HLS Leukocyte Data Performed by ToxStrategies

Endpoint	**T-Test**					**Trend Tests**	
Dose, mg/kg/day (n)	**0 (10)**	**2.9 (10)**	**10.6 (9)**	**42.0 (9)**	**191.1 (10)**	**Jonckheere[1]**	**Jonckheere[2]**
Total WBCs[3]	7.97 (2.213)*	7.763 (2.653)	5.41 (1.392)	5.53 (1.756)	4.54 (1.019)		
p-values		0.76	0.008	0.016	0.001	0.00013	0.00002
Lymphocytes	6.98 (2.146)	6.36 (2.452)	4.39 (1.308)	4.63 (1.564)	3.73 (0.941		
p-values		0.56	0.006	0.014	0.001	0.00006	0.00001
Basophiles	0.01 (0.006)	0.01 (0.006)	0 (0.005)	0 (0.007)	0 (0.004)		
p-values		0.44	0.018	0.046	0.001	0.00062	0.00011
Monocytes	0.22 (0.08)	0.23 (0.119)	0.13 (0.053)	0.13 (0.040)	0.10 (0.040)		
p-values		0.94	0.012	0.008	0.001	0.00018	0.00003
LUCs	0.11 (0.040)	0.11 (0.056)	0.06 (0.023)	0.06 (0.026)	0.04 (0.019)		
p-values		0.93	0.011	0.007	0.001	0.00002	0.000003

* all results shown represent Cell Count x 10^9, Mean (s.d.)

[1] Jonckheere 2-sided test (http://www.biostat.wustl.edu/archives/html/s-news/2000-10/msg00126.html)

[2] Jonckheere 1-sided test for decreasing trend (http://tolstoy.newcastle.edu.au/R/help/06/06/30112.html)

[3]WBC-white blood cells; LUCs-large unstained cells

In addition to statistical significance, it is also important to determine if the effects are biologically meaningful. To accomplish this, the WBC counts in the HLS sulfolane drinking water study were compared to historical control data for the same species, strain, gender, and age animals from the same HLS laboratory over the same time period. Because the historical control group is comprised of many more animals than in the concurrent control group (N=393 vs 10, respectively), the historical control dataset provides a much more

robust dataset for establishing the normal range of variability for the endpoints of interest. Blood cell counts from individual female rats in the HLS sulfolane drinking water study were converted to incidence counts using the historical control ranges. These incidence counts were then compared using Fisher's Exact Test with Holm's correction for multiple comparisons. There were no significant differences between dose groups and the control. This finding suggests that though statistical analyses indicate a treatment-related decrease in WBC counts at the three highest dose levels tested in female rats, the effects appear to be subtle as they are not outside of the historical control range for the specific species, strain, gender, age, and laboratory. This conclusion is consistent with that of the HLS study authors conclusion that the toxicological significance of the WBC effects was unclear due to the lack of evidence of any chronic inflammatory change or compromised immune function in female rats, as well as lack of any effects on bone marrow, thymus or spleen that might account for reduced numbers of white blood cells.

Despite this uncertainty regarding the toxicological significance of the WBC effects, based on apparent trend in the data and aforementioned statistical tests, we modeled the total WBC, lymphocyte, monocytes, and large unstained cells (LUCs) cell count data (a continuous variable) using the U.S. EPA Benchmark Dose Software (BMDS). Basophils were not modeled because the mean values for the control and four dose groups were 0.01, 0.01, 0.00, 0.00 and 0.00×10^9 cells/L.

Initially, none of the models in the BMDS were able to reasonably fit the total WBC, lymphocyte, monocyte, or LUC cell count data. In such instances, risk assessors sometimes drop the highest dose in the study and remodel the data to improve the model fit. However, this should only be done if there is evidence of lethality at the highest dose level or there is evidence of a plateau in the toxic response at the highest dose level. As this is not the case with the HLS dataset, there is no scientifically supportable basis for dropping the highest dose level solely for model fitting purposes. However, the dose spacing in the HLS study was such that the two lower doses covered only a small proportion (10.6 / 191.1 = 5.5%) of the total dose range, and thus the higher doses unduly influence the model fit. A scientifically supportable approach for addressing a situation like this is to log transform the doses. By log transforming the doses, the lower doses take on a more even spacing (2.5/5.3 = 47%), and lowers the influence of the high dose without arbitrarily dropping data points.

Log transformation of dose has previously been used to model a reduction in lymphocytes in the toxicological review of the noncancer effects of benzene (U.S. EPA, 2002b). Therein, RfC and RfD values were established using lymphocyte count data from humans exposed to benzene via inhalation. All of the continuous models from the BMDS produced poor fits to the benzene data, in part, due to the supralinear response pattern. Therefore, the EPA log transformed the doses, and remodeled the untransformed responses against transformed doses. Specifically, the dose transformation is ln(dose+1), which produces a transformed dose for the unexposed controls of ln(0+1)=0. This dose transformation resulted in models that fit the data, and EPA stated, "the linear model was selected because it is the most

parsimonious." The resulting BMD and BMDL values were then converted back to arithmetic dose as follows: $e^{\ln(dose+1)} - 1$.

Applying the same approach to the total WBC and lymphocyte data from the HLS study, along with U.S. EPA's default approach of using a benchmark response (BMR)[2] based on 1 standard deviation from the concurrent study controls and fitting a linear model, we computed default $BMDL_{1SD}$ values of 11.9 and 14.5 mg/kg/day based on total WBC and lymphocyte counts, respectively (with acceptable p-values)[3]. Using the same approach, models were unable to achieve reasonable fits to the cell counts for monocytes or LUC. As such, these two endpoints were not considered further in our analyses. This was determined to be inconsequential since all hematological endpoints exhibited the same LOAEL in female rats and total WBC counts were observed to be most dependent on lymphocyte counts in the HLS dataset for female rats.

When modeling continuous datasets, U.S. EPA has indicated that the data can be modeled using the historical standard deviation from control (i.e. untreated) animals along with the concurrent control mean data from the study of interest when such data is available (U.S. EPA, 2000). In contrast to the limited number of female rats in the concurrent control group in the HLS sulfolane study (N=10), historical control data can provide a better indication of the true variability of a given biochemical or toxicological endpoint. As such, we obtained historical control hematology data for 393 female CD Sprague-Dawley rats of 16-21 weeks of age from HLS. This historical control data is ideal because it comes from the same species, strain, sex, and age group of animals from the same laboratory and time period as in the sulfolane study; and moreover, because these data come from the same HLS laboratory, the total WBC and lymphocyte counts were most likely obtained using the same collection and analytical techniques as were used in the sulfolane study. Therefore, the HLS historical control data provides a much more robust dataset for establishing the normal range of variability for the endpoints of interest. Given this, the WBC and lymphocyte standard deviations from the concurrent HLS control animals (2.213 and 2.146, respectively) were replaced with those from the historical HLS dataset (2.626 and 2.290, respectively), and BMD modeling was again performed with the BMR set to 1 standard deviation. In accordance with this approach, the $BMDL_{1SDh}$ values for total WBC and lymphocytes were determined using a linear model. Because the standard deviations from the historical data were slightly greater than those of the HLS study, the resulting BMD and BMDL values increased slightly compared to those calculated using the concurrent control standard deviation. These BMDL values were **15.1** and **16.0** mg/kg/day for WBC and lymphocytes, respectively (**Table 2**). However, because this historical standard deviation is drawn from a much larger sample size (393 vs 10), the historical standard deviation is a more representative measure of the true variability in total WBC and lymphocyte counts. As such, the BMD and BMDL values derived based on the historical

[2] It should be noted that for approximately normally distributed data, the dose that produces a shift of the mean response, from the control mean, equivalent in size to one standard deviation (BMR=1 SD) results in approximately an additional 10% of the animals with abnormal levels of the biological endpoint under study.

[3] We also considered that the data may be lognormally distributed; however, Pearsons's index of skewness did not indicate that the data were strongly skewed.

control standard deviation are more defensible scientifically than are those derived using the standard deviation based on the limited number of concurrent study controls. The BMD model output for this analysis is provided in **Appendix C**.

Table 2

Summary of BMD Modeling Results for HLS (2001) Based on Historical Control Standard Deviation

Model Parameter	Endpoint: Reduced Cell Counts	
	WBC	**Lymphocytes**
p-values	0.1677	0.158
scaled residual	0.168	0.232
BMD[1], ln(dose + 1)	4.22	4.34
BMDL, ln(dose + 1)	2.78	2.83
BMD	67.03	75.71
BMDL	15.12	15.95

[1] Because the doses were log transformed, the BMD and BMDL values reported in the BMD software output were ln(dose + 1) and were mathematically converted back to arithmetic scale for reporting BMD and BMDL values

The BMD and BMDL values reported in **Table 2** reflect results obtained when fitting a linear model and were ultimately chosen as the PODs based on considerations of model fit, as well as on the basis of parsimony. The p-values in **Table 2** are testing the goodness-of-fit of a model to the experimental dose-response data; a small p-value rejects the fit of the model and thus p-values should exceed 0.10. The scaled residual also measures model fit, and is essentially a measure of the difference between an observed mean value at a given dose and the predicted mean at the same dose. These values should not be greater than 2 or less than -2. The choice of model based on parsimony is consistent with the approach taken by U.S. EPA in their selection of results from the linear model in their BMD modeling of the effects of benzene on lymphocytes (U.S. EPA, 2002b). A comparison between these BMD and BMDL values to those obtained with other models and modeling approaches is provided in **Appendix C**.

It is also informative to compare hematological data from inhalation studies with sulfolane to the data from HLS (2001). In this regard, inhaled doses employed in the Andersen et al. (1977) study can be converted to an equivalent mg/kg/day dose. Although these values are rough estimates, they can be compared to the oral exposure doses in the HLS study. Specifically, Andersen et al. (1977) examined the effects of sulfolane in several species for various durations of exposure. A previous study by Andersen et al. (1976) showed that

sulfolane is rapidly distributed systemically and the LD_{50} does not vary greatly by exposure route – perhaps suggesting that the toxicity is not route-dependent. Using the following formula, estimates of inhaled doses can be made from the inhalation concentrations in the Andersen et al. (1977) study:

$$\text{Conc} \times \text{MV} \times \text{Time} \div \text{BW},$$

where MV is minute volume (L/min) and BW is bodyweight (kg)[4].

After 95 days of exposure to 20 mg/m^3 sulfolane 23 hr/day, Andersen and colleagues reported no adverse effects in rats (males and females), guinea pigs (males and females), dogs (males), or monkeys (males). For rats, 20 mg/m^3 is roughly equivalent to 23 mg/kg/day (0.02 mg/L × 0.21 L/min × 60 × 23 ÷ 0.25 kg). Although data from the Zhu et al. (1987) 90-day study provided some indications that guinea pigs might be more sensitive than rats to reduced WBC, Andersen et al. (1977) reported a NOAEL for effects on WBC counts in guinea pigs at 159 mg/m^3; which roughly equivalent to 75 mg/kg/day in guinea pigs. Although these route conversions are only estimates, they provide additional support that the $BMDL_{1SDh}$ of 15.1 mg/kg/day for reduced WBC count in female rats is a conservative POD even for the putatively sensitive guinea pig.

3.1.1.2 Zhu et al. (1987)

Zhu et al. (1987) performed 90-day and 6-month oral toxicity studies in rats and guinea pigs. None of the results from the 90-day study were amenable to statistical analyses or BMD modeling (see Section 2.2). In the 6 month study, guinea pigs were exposed to doses of 0.25, 2.5, 25, and 250 mg/kg/day sulfolane. Some toxicity endpoints were measured in some animals after only 90 days of exposure; however, these data were not modeled or considered for risk assessment because measures of variability were not provided in the study and thus not amenable to statistical analyses or BMD modeling (see Section 2.2). Incidence data for pathological effects in the spleen were not modeled at 90 days because similar data were presented with a larger sample size of animals at 6 months (14 animals vs 22-25 animals per dose group). After 6 months of exposure, Zhu et al. (1987) reported incidence data for fatty degeneration (steatosis) of the liver, severe fatty degeneration of the liver, and dispersion of the white pulp of the spleen. The incidences for these effects are summarized below in **Table 3**. All three endpoints were positive for association and trend. Pair wise comparisons using the Fisher's exact test with Holm's correction for multiple comparisons revealed that the incidences in the highest dose groups were statistically different from control ($p < 0.05$, **Table 4**), whereas the incidence in all other dose groups was not statistically different from controls (**Table 4**). Fisher's exact test with Holm's correction for multiple comparisons is the appropriate test because it allows for fewer errors in statistical inference when multiple tests are conducted on the same family of data as a whole and is thus a more accurate way to judge differences among the dose groups. Therefore, using a default LOAEL/NOAEL approach, the LOAEL and NOAEL values for these endpoints would be 250 and 25 mg/kg/day, respectively.

[4] Standard bodyweights of 0.25 and 0.5 kg were used for rats and guinea pigs (U.S. EPA, 2002b), and minute volumes of 0.21 and 0.17 L/min (U.S. EPA, 1988).

Table 3
Data Summary for Effects Observed in Guinea Pigs Exposed Orally for Six Months (Zhu et al.,1987)

Dose (mg/kg)	Spleen	Fatty Liver	Severe Fatty Liver	Bone Marrow Cell Count[a]
0	0/25	0/25	0/25	$16.43 \times 10^4/mm^3$
0.25	0/22	0/22	0/22	n.d.[b]
2.5	2/26	2/26	1/26	$10.99 \times 10^4/mm^3$
25	2/25	4/25	2/25	$12.25 \times 10^4/mm^3$
250	7/22	7/22	5/22	$10.56 \times 10^4/mm^3$

[a] cell counts were only provided for animals exposed for 3 months; note: no measure of variability was provided
[b] cell count at this dose was characterized as "not different from control"

Table 4
Statistical Evaluation of Incidence Data in Zhu et al. (1987)

	p-values					
	Spleen		Fatty Liver		Severe Fatty Liver	
Dose (mg/kg)	FET*	FET with Holm's correction	FET	FET with Holm's correction	FET	FET with Holm's correction
0.25	1	1	1	1	1	1
2.5	0.26	0.73	0.25	0.51	0.51	1
25	0.25	0.73	0.055	0.16	0.24	0.73
250	**0.0027**	**0.011**	**0.0027**	**0.011**	**0.017**	0.069

*Fisher's Exact Test (FET) was performed with and without Holm's correction for multiple comparisons; bolded values indicate statistically significant at $p \leq 0.05$. All three endpoints were also positive for trend test.

BMD modeling was used to determine POD values for each endpoint. **Appendix D** contains the output from the models that provided the best fit to the incidence data. Log-logistic models provided the best fits to the data (higher p values, lower AIC values, and scaled residuals less than absolute value of 2). These models also happened to provide the lowest BMDL values for the three endpoints. Across the three endpoints, fatty liver (steatosis) provided the lowest, most health protective $BMDL_{10}$ value (**22.6 mg/kg/day) (Table 5)**. It should be noted that in modeling these data the slope was restricted to be ≥ 1 in accordance with U.S. EPA guidance (U.S. EPA, 2000). This is done to prevent the estimated dose response curve from taking on a biologically implausible very steep slope as the dose approaches zero, resulting in a very improbable low estimate of the true BMD. Said differently, it is unlikely that very low doses cause dramatic increases in response relative to higher doses. It is also worth noting that restricting or not restricting the slope is not an issue for some mathematic models in the BMDS. For fatty liver, other such models (e.g.

multistage) fit the data reasonably well but gave higher estimates of the BMDL. Finally, risk assessors sometimes drop the high dose results of a study if high-dose toxicity is apparent (e.g. mortality) of if the response has clearly reached a plateau. However since this was clearly not the case with this particular dataset, the high dose was retained in our analysis.

Table 5
Comparison of BMD Modeling Results for Incidence (Zhu et al., 1987)

Parameter or Value	Spleen	Fatty Liver	Severe Fatty Liver
Model	Log Logistic	Log Logistic	LogLogistic
BMD_{10}	58.9	48.5	83.6
$BMDL_{10}$	28.3	**22.6**	38.1
P-value	0.329	0.172	0.544
Residual	0.237	1.37	0.814

3.1.1.3 OECD (2004)

The IUCLID file (OECD, 2004) provided detailed results from a GLP OECD reproductive/developmental toxicity screening study. We were able to confirm the tabular data in the IUCLID file by obtaining the original report online via the ACToR database (http://actor.epa.gov/actor/faces/ACToRHome.jsp). Although the original study was in Japanese, the tabular data was in English. In this study, male and female rats were exposed to 60, 200 and 700 mg/kg sulfolane daily by oral gavage for 14 days prior to mating. Females continued to be exposed via gavage throughout gestation and through day 3 of lactation. At 200 mg/kg/day, birth index (live pups born divided by number of implantation scars ×100) and the number of pups alive at day 0 and day 4 of lactation were decreased; therefore, the study authors reported a NOAEL of 60 mg/kg/day. Although neither the number of implantation scars nor live pups born were significantly reduced at any dose level, the ratio ×100 (i.e. birth index) was significantly reduced. Therefore, we modeled this more sensitive endpoint, as well as the number of live pups on day 4 of lactation because it includes the longest exposure duration for each fetus/pup (i.e. includes pre- and post-natal exposure). As described above for our analyses of the HLS (2001) dataset, these continuous data were modeled using a BMR of 1 standard deviation. The best models fits for these data provided $BMDL_{1SD}$ values ranging from 96 to 161 mg/kg/day (**Table 6**); the BMD modeling of these data are presented in **Appendix E**. Based on overall fit, 161 mg/kg/day was identified as the POD for live pups on day 4 and 120 mg/kg/day for birth index.

Table 6
Comparison of BMD Modeling Results for Live Pups Day 4 and Birth Index

Model Parameter				
	Live Pups on Day 4			
Model	Exponential	Polynomial	Power	Linear
BMD_{1SD}	239.4	255.8	248.2	(poor fit)
$BMDL_{1SD}$	161.2	149.4	153.1	
AIC	114.9	115.0	114.9	
P-value	0.7113	0.6229	0.6568	0.026
Residual	0.21	0.33	0.25	
	Birth Index			
Model	Exponential*	Polynomial	Power	Linear
BMD_{1SD}	214.9	219.9	216.7	142.6
$BMDL_{1SD}$	119.71	114.2	117.36	95.7
AIC	228.7	229.0	228.8	228.4
P-value	0.5824	0.458	0.5518	0.2768
Residual	0.5669	0.661	0.594	0.122

* two Exponential submodels provided BMDL values of 88.5 mg/kg with poorer p- values (0.183)
note: lower AIC (Akaike's Information Criterion) values indicate a better fit of the model to the data

3.1.1.4 Chronic RfD Derivation

Risk assessors have argued for at least two decades that the LOAEL/NOAEL approach is an inferior risk assessment approach because it is limited to the doses tested, does not appropriately address study size, does not allow for direct comparisons across studies and endpoints based on a common response level (e.g. 10% increased risk), and can inappropriately reward poorer studies with less statistical power to detect effects resulting in higher LOAEL and NOAEL values (Crump, 1984; Leisenring and Ryan, 1992; Gaylor et al., 1998; Allen et al., 1998; U.S. EPA, 2000, 2002a). In contrast, BMD modeling has been recognized as the preferred alternative because it takes into account the shape of the dose-response curve, the confidence limits reflect the size of the study, and allows comparison of comparable results across studies and endpoints at any response level (e.g. 10% increased risk) (Allen et al., 1998; U.S. EPA, 2000, 2002a). Therefore, consistent with U.S. EPA (2002a), we modeled several endpoints using U.S. EPA's BMDS. **Table 7** contains a summary of POD values obtained using the default LOAEL/NOAEL approach and BMD modeling approach. Human equivalent concentration (HEC) values for these PODs were calculated using bodyweight ¾ scaling as follows:

$$\text{HED dose} = \text{POD mg/kg-day} / (70\text{kg}/BW_A)^{0.25},$$

where BW_A is the standard bodyweight for guinea pigs or rats of 0.5 and 0.25 kg, respectively (U.S. EPA, 2002a). These values were then adjusted by various UFs based on the following rationale. For the Zhu and HLS studies, the usual 10-fold interspecies UF_A was reduced to 3-fold due to the use of bodyweight ¾ scaling which is intended to account for potential species differences in toxicokinetics. As such, the 3-fold UF_A is to account for uncertainties relating to interspecies differences in toxicodynamics. For the reproductive and developmental toxicity study, the full 10-fold UF_A was used because of the complication of the change in dam weight and physiology during gestation.

In the HLS study, the effects on WBC counts were only observed in female rats. Therefore, the intraspecies UF_H (also called the human variability factor) was reduced from 10 to 3 because a potentially sensitive population (i.e. females) is accounted for in the POD. In contrast, the effects in the Zhu study were not provided for males and females separately; therefore, a full 10-fold UF_H was applied to be conservative. Similarly, a full UF_H was used for potential parental (males and females were exposed) and pup variability in reproductive and developmental toxicity study. The HLS (2001) study was a 90-day drinking water study, therefore a 10-fold UF_S was applied for extrapolating the results of this 90-day subchronic study to a chronic duration. In contrast, Zhu et al. (1987) was a 6-month study and, as such, is officially categorized as a chronic study[5], and thus an UF_S of 1 was considered. However, because of the potential uncertainty associated with the lack of full lifetime exposure, a 3-fold UF_S was applied. Consistent with U.S. EPA practices, a UF_S was not applied for developmental studies (U.S. EPA, 2002a). For the database UF (UF_D), a value of 3 was chosen for several reasons. A default UF_D of 10 is typically applied if there is an absence of both developmental toxicity studies and a two-generation reproduction/developmental study. In the sulfolane database, there are two developmental toxicity studies and a preliminary reproductive/developmental toxicity study for sulfolane. The BMD modeling of one of these studies and the NOAEL values from two of the studies indicate relatively mild effects at doses much greater than those where effects on liver or white blood cells were observed. Moreover, studies indicate that sulfolane is not likely to be mutagenic. The lack of a full 2-year cancer bioassay is a limitation. However, a cancer study performed by NCI reported no dose-dependent carcinogenic effects following chronic exposure to sulfolene, a compound that is very similar structurally to sulfolane. Based on the lack of two-generation reproduction/developmental study, a 3-fold UF_D was applied.

[5] The U.S. EPA IRIS website defines a chronic study as "A toxicity study designed to measure the (toxic) effects of chronic exposure to a chemical"; and defines chronic exposure as "Repeated exposure by the oral, dermal, or inhalation route for more than approximately 10% of the life span in humans (more than approximately 90 days to 2 years in typically used laboratory animal species)."

Table 7
Potential Oral RfDs Derivations for Various Endpoints

Duration	Effect (mg/kg/day)	POD	HEC	UFs[1]					RfD[2] (mg/kg/day)
				A	H	S	D	Tot.	
Repro. & Develop. Toxicity	birth index	$BMDL_{1SD}$ = 120	NA	10	10	1	3	300	0.40
		NOAEL = 60	NA	10	10	1			0.20
Sub-chronic (90 days in rats)	Reduced WBC count[3]	$BMDL_{1SD}$ = 15.1	3.7	3	3	10	3	300	**0.012 (0.01)**
	Reduced WBC count (incidence)	$BMDL_{10}$ = 21.8	5.3	3	3	10			0.018 (0.02)
	Reduced WBC count	NOAEL = 2.9	0.71	3	3	10			0.0024 (0.002)
Chronic (6 months in guinea pigs)	spleen	$BMDL_{10}$ = 28.3	8.2	3	10	3	3	300	0.027 (0.03)
	fatty liver	$BMDL_{10}$ = 22.6	6.6	3	10	3			0.022 (0.02)
	sever fatty liver	$BMDL_{10}$ = 38.1	11.1	3	10	3			0.037 (0.04)
	fatty liver	NOAEL = 25	7.3	3	10	3			0.024 (0.02)

[1]UF = uncertainty factor, UF_A = interspecies UF, UF_D = database UF, UF_H = intraspecies UF, UF_S = subchronic to chronic UF

[2] values in parentheses are rounded values

[3] this BMDL is based on historical control data from the HLS laboratory

3.1.1.4.1 Selection of POD and RfD

In the three studies described above, there were no statistically-derived NOAEL values for endpoints that could not also be modeled by BMD analysis. Because BMD modeling approaches are considered better than LOAEL/NOAEL approaches (see Section 3.1), the potential RfD candidates based on BMD modeling were considered for selecting the final RfD. Overall, the BMD analyses summarized above (**Table 7**) provide a narrow range of potential POD values that can be used to develop an oral RfD for sulfolane, suggesting generally good agreement between the different modeling approaches. Changes in WBC counts in the HLS (2001) appear to represent the most sensitive endpoint; however as already discussed above, the adversity of this effect remains to be demonstrated. Nonetheless, in the interest of being conservative (i.e., health protective), these endpoints were treated as if they were "adverse" for purposes of developing a POD for risk assessment purposes even though this has not been clearly demonstrated by the available data. Notably, the HLS (2001) was also a GLP-conducted study. Given the much larger sample size for historical vs concurrent control animals (393 vs 10, respectively), the historical standard deviation is believed to be a better measure of variability in WBC and lymphocyte counts in untreated animals. As already noted, the linear model provides the best fit and is the most parsimonious and, as such, BMDL values from this model were selected as the PODs. Examination of **Table 2** indicates that the BMDL from the linear model for total WBC was slightly lower than that for lymphocytes. Therefore, the

recommended POD is based on the $BMDL_{1SDh}$ for decreases in total WBC of **15.1 mg/kg/day**. As shown in **Table 7**, this results in an RfD value, **0.012 mg/kg/day** (rounds to 0.01 mg/kg/day). This value is recommended as the RfD.

3.1.2 Inhalation Exposure Route

3.1.2.1 Chronic RfC

Consistent with the CCME (2006a), Andersen et al. (1977) was identified as the most relevant inhalation study for sulfolane. Andersen et al. (1977) exposed rats, guinea pigs, dogs and squirrel monkeys to 2.8, 4.0, 20, 159, and 200 mg/m^3 aerosolized sulfolane. It should be noted that at 159 mg/m^3 only guinea pigs were studied, and at 200 mg/m^3 rats were not examined. Based on the findings of this study, Andersen et al. (1977) reported a NOAEL of 20 mg/m^3 for all four species. Effects observed at the higher doses (159 - 200 mg/m^3) included death, convulsion, fear and aggression, motor disturbances, seizures, chronic lung inflammation, pleuritis, lung hemorrhage (although no alterations in breathing were observed), and hematological changes. Considering that no such effects were observed in the four species at 20 mg/m^3, this level represents a fairly robust NOAEL. It should also be noted that empirical modeling approaches (e.g. benchmark dose analysis) could not be performed with these data because Andersen et al. conducted several different studies, with variations in the species, sexes, and durations across the species. The chronic RfC was calculated as follows and is summarized in **Table 8**:

$$20\ mg/m^3 * 23/24 * 7/7 = 19.2\ mg/m^3 \text{ (duration adjusted)}$$

This dose was then divided by 1,000 based on rounding of the following UFs: (UF_A = 10, UF_H = 10, UF_S = 3, and UF_D= 3). The RfC was calculated as follows:

$$(19.2\ mg/m^3) / (1{,}000) = 0.0192\ mg/m^3 \text{ (rounds to } 0.02\ mg/m^3)$$

The rationale for a 10-fold UF_A was that no interspecies adjustment to dose was made; the 10-fold UF_H was to account for potential human variability in susceptible populations. The rationale for a 3-fold UF_S was that while the study was only of 90-day (subchronic) duration, unlike most subchronic studies that involve exposure for a few hours per day for 5 days per week, these animals were dosed 23 hr/day for 85-90 days. Moreover, as noted elsewhere (CCME, 2006; Andersen et al., 1977), the low volatility of sulfolane suggests that chronic exposure to sulfolane is unlikely. In this regard, Andersen et al. (1977) had to wrap the reservoir and input lines to the chamber with heat tape in order to maintain the chamber temperature because sulfolane freezes at 27.5 °C. A 10-fold UF_D was considered. However, the oral database for sulfolane suggests limited reproductive and developmental toxicity, limited evidence for mutagenicity, and that sulfolane is unlikely to be carcinogenic (based on sulfolene). Moreover, the NOAEL value from Andersen et al. (1977) is for four species (including primates), which suggests that this value is likely protective for humans. And finally, the low volatility of sulfolane means that sustained inhalation exposure is unlikely to occur and that the subchronic exposures performed by Andersen and colleagues is the only likely exposure scenario that needs to be studied (i.e. the lack of chronic

inhalation studies is not a major health concern). Nevertheless, Andersen et al. (1977) state that there was a trend in increasing sensitivity progressing from rodents to dogs to monkeys for CNS effects, perhaps suggesting greater human sensitivity. Therefore a 3-fold UF_D was applied.

3.1.2.2 Acute RfC

As noted by British Columbia Ministry of Water, Land, and Air Protection in their water quality guidelines for sulfolane (BCMWLA, 2003), there are no useful acute studies for sulfolane. The acute studies performed by Andersen et al. (1977) were at very high concentrations and caused overt toxicity. For example, all nine rats exposed to 3,600 mg/m^3 sulfolane for 17.5 hours had convulsions and were *in extremis*. Two monkeys exposed to 4,850 mg/m^3 for 18.5 hours convulsed and vomited during exposure. In separate experiments in the same study, rats, guinea pigs, dogs and monkeys were exposed to 495 mg/m^3 for 8 hour per day for 27 days. Of the nine exposed monkeys, three died and six were found *in extremis*. Because no suitable PODs were found from acute or subacute exposures, the same 20 mg/m^3 POD used for chronic RfC derivation was selected as the POD for acute RfC derivation. In this instance, no duration adjustment or UF_D was applied as per recommendations in U.S. EPA (2002a). Considering that 20 mg/m^3 is protective of 90-day exposures in four species, and that a POD for a 90-day study is likely to be health protective for a 24-hr acute value, the UF_A was set to 3. It is also noteworthy that the exposure conditions used in Andersen et al. (1977) were truly continuous (i.e. 23 hr/day). Thus, the POD was reduced by a 3-fold UF_A and 10-fold UF_H:

$$(20\ mg/m^3) / (30) = 0.67\ mg/m^3$$

3.2 Recommended Toxicity Factors

Based on the analyses described above in Sections 3.1.1 and 3.1.2, we recommend the following oral and inhalation toxicity values for sulfolane (**Table 8**):

Table 8
Summary of Recommended Toxicity Factors for Sulfolane

	Point of Departure	Duration Adjusted POD	Uncertainty Factors (UF: A, H, S, D)	Toxicity Factor
Chronic Oral RfD				
ToxStrategies[a]	3.7 mg/kg-d	NA	300 (3, 3, 10, 3)	0.01 mg/kg-d
Chronic Inhalation RfC				
ToxStrategies[b]	20 mg/m^3	19.2 mg/m^3	1,000 (10, 10, 3, 3)	0.02 mg/m^3
Acute Inhalation RfC				
ToxStrategies[b]	20 mg/m^3	NA	30 (3, 10, 1, 1)	0.67 mg/m^3

UF = uncertainty factor; UF_A = interspecies UF; UF_D = database UF; UF_H = intraspecies UF; UF_S = subchronic to chronic UF

[a]Based on HLS (2001).

[b]Based on Andersen et al. (1977).

3.3 Derivation of Toxicity Factors by Others

Although toxicity factors were not available for sulfolane from the common sources of available toxicity factors (i.e., U.S. EPA's Integrated Risk Information System, U.S. EPA's Provisional Peer-Reviewed Toxicity Values, U.S. EPA's National Center for Environmental Assessment provisional values, the State of California's toxicity databases, and the Agency for Toxic Substances and Disease Registry's Minimal Risk Level list), a chronic oral tolerable daily intake (TDI) of 0.0097 mg/kg-day (rounded to 0.01 mg/kg-day) was identified in documents describing development of water quality guidelines developed by the BCMWLA (2003) and CCME (2006a). This TDI is based on the subchronic drinking water study in rats conducted by Huntingdon Life Sciences (HLS, 2001) that has already been described above in Section 2.2. The rationale CCME provided for their choice of study was based on the fact that the study employed an oral exposure as opposed to the inhalation exposures described in Andersen et al. (1977). HLS (2001) was chosen over the oral study conducted by Zhu et al (1987) as CCME determined that the uncertainties in the interpretation of some of the toxicological endpoints and the lack of available data to confirm that "good laboratory practice" (GLP) had been followed in this study. However, it is unclear if this determination was based on the lack of availability of an English translation or based on a determination regarding a specific shortcoming in the study. In developing the TDI, CCME used the default NOAEL approach and applied a 100-fold margin of safety to account for inter- and intra-species differences in toxicokinetics and toxicodynamics. An additional 3-fold safety factor was applied to account for the following: i) incomplete database, ii) chronic studies are lacking, and iii) not all potentially adverse effects have been identified. As already discussed above in Section 3.1, there are a number of limitations of the default NOAEL approach including: a) the LOAEL/NOAEL values are limited to the doses tested, b) the LOAEL/NOAEL does not appropriately reflect study size, c) the LOAEL/NOAEL values cannot be directly compared across studies and endpoints based on a common response level (e.g. 10% increased risk), and d) the approach can inappropriately reward poorer studies with less statistical power to detect effects resulting in higher LOAEL and NOAEL values.

Since the time of release of our initial report summarizing the available toxicity information for sulfolane (i.e., *Assessment of Toxicological Data for Sulfolane – Update, November 23, 2009*), the Alaska Department of Health and Social Services requested that the Agency for Toxic Substances and Disease Registry (ATSDR) conduct a health consultation and develop a public health action level for sulfolane. In contrast to CCME (2006a), ATSDR established a much lower chronic oral toxicity value of 0.0025 mg/kg/day for sulfolane (ATSDR, 2010). This value was derived by dividing the no observable adverse effect level in guinea pigs of 0.25 mg/kg/day identified by Zhu et al. (1987) by 10-fold uncertainty factors each for inter- and intra-species variability. There are a number of shortcomings associated with the ATSDR analysis. The 0.25 mg/kg/day NOAEL used by ATSDR appears to be based on the conclusions of the study authors, and it does not appear that ATSDR attempted to statistically analyze, or otherwise model, the data to derive their own POD values. If one were to use a LOAEL/NOAEL approach based on the data from Zhu et al. (1987), statistical analyses should be performed on the data. As was shown in **Table**

4, the incidence for effects in the spleen and liver were positive for trend. However, pair wise comparisons using the Fisher's exact test with Holm's correction for multiple comparisons revealed that only the highest dose resulted in a statistically significant increase relative to controls. As a result, this dose level (250 mg/kg/day) is the LOAEL in guinea pigs in this study, while the next highest dose level (25 mg/kg/day) is the NOAEL. Importantly, the incidence for pathological effects in the 2.5 mg/kg/day dose groups were not statistically increased with or without Holm's correction; therefore, the use of 2.5 mg/kg/day as a LOAEL and 0.25 mg/kg/day as a NOAEL is clearly inappropriate by any standard statistical analysis.

Another shortcoming in the ATSDR report is that it is not quite clear which endpoint from Zhu et al. (1987) was selected as the key finding for risk assessment. ATSDR noted that effects were observed in hepatic and lymphoreticular systems of rats (90-days) and guinea pigs (90-days and 6 months). Zhu et al. provided no mean and standard deviation or incidence data from their 90-day study. In the 6-month study in guinea pigs, incidences for histopathological findings were provided, but other quantifiable measures for independent dose-response analysis were not provided. Thus, ATSDR has apparently based their drinking water recommendations on a subjective inspection of the incidence rates and other semi-quantitative values (e.g. mean values with no measures of variability). In this regard, changes in serum levels of hepatic enzymes and bone marrow counts were not provided along with standard deviations. Moreover, reference ranges needed to assess the biological relevance of these changes were also not provided. Thus, the biological significance of many of the effects reported in Zhu et al. (1987) is uncertain.

Additionally, similar to the TDI developed by BCMWLA (2003) and CCME (2006a), the chronic oral toxicity factor developed by ATSDR is based on the default NOAEL approach. As already discussed above regarding the TDI, as well as in Section 3.1, there are a number of significant limitations of the default NOAEL approach and, as such, USEPA and others prefer to use benchmark dose modeling whenever possible as this approach allows one to consider the shape of the dose response curve and use all of the data when developing a POD.

Finally, we also located a "permitted daily exposure" (PDE) value for sulfolane. According to a technical guideline entitled "International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use" (ICH, 2005), a PDE is defined as a "pharmaceutically acceptable intake of residual solvents." Although it is not synonymous with TDI, ADI, or RfD values, its intent is to place residual solvent levels in manufactured pharmaceutical products into context of what might be considered a safe daily exposure level. Because these solvents cannot be completely eliminated during the manufacture of beneficial pharmaceuticals, it should be recognized that in the context of disease treatment or prevention there are tradeoffs regarding the presence of these solvents. Nevertheless, the PDE is calculated in a fashion similar to other risk values, where NOEL or LOEL values from a study are divided by several adjustment factors that take into account differences between laboratory animals and humans, human variability, severity of effects, and other study specific elements such as duration. In ICH (2005), a PDE of 1.6 mg/day was recommended for sulfolane. This was reported to be the lowest of at least five

calculated PDE values based on a NOEL in rats from a developmental toxicity study conducted by Glaxo Wellcome, and two NOEL values each in guinea pigs and rats from the inhalation study conducted by Andersen et al. (1977). These studies were previously discussed in Sections 2.2 and 2.3. In accordance with the PDE methodology, the PDE was derived using a standard human bodyweight of 50 kg (therefore 1.6 mg/day is equivalent to 0.032 mg/kg/day). Using the 70 kg bodyweight that is often used in risk assessment conducted by agencies such as the U.S. EPA, this is equivalent to 0.023 mg/kg/day. This PDE value is a little over 2-fold higher than the TDI recommended by CCME and the RfD recommended by ToxStrategies. Like the approaches implemented by CCME and ATSDR, the LOEL/NOEL approach by ICH (2005) has inherent shortcomings. Furthermore, the extrapolation from inhalation studies by Andersen et al. (1977) to derive oral intake values has inherent uncertainties.

As was described in Section 3.0, the above LOAEL/NOAEL approaches employed by these organizations have methodological shortcoming relative to BMD modeling approaches described in Section 3.1. Nevertheless, results based on these quantitative dose-response modeling approaches suggest that the TDI previously proposed by CCME is a reasonably health protective value for exposure to sulfolane in drinking water. In the following section, the application of the RfD proposed by ToxStrategies in setting tap water screening values is described and compared to other approaches.

4.0 Derivation of Tap Water Screening Levels

To date, the USEPA has not established a maximum contaminant level (MCL) for sulfolane. Therefore, ToxStrategies developed tap water screening levels for sulfolane, based on the toxicity factors derived in Section 3.1 of this white paper. As already discussed, the toxicity factor for the drinking water ingestion pathway is based on a subchronic GLP drinking water study and application of state-of-the-science BMD modeling and, as such, is believed to represent the most scientifically defensible value derived to date. Nonetheless, for comparison purposes, tap water/drinking water screening values based on toxicity factors developed by other entities are also presented.

4.1 Tap Water Screening Levels Derived by ToxStrategies

4.1.1 Drinking Water Ingestion

ToxStrategies calculated drinking water screening levels based on the ingestion pathway equations and assumptions presented with U.S. EPA's Regional Screening Levels (RSLs) (U.S. EPA, 2009b) and the most conservative chronic RfD calculated using BMD modeling (0.01 mg/kg-day based on HLS (2001). As already discussed, the HLS (2001) study is the most robust, reliable data source currently available to for deriving oral reference values.

Per USEPA (2009b), tap water RSLs for noncarcinogenic compounds are based on an adult scenario (e.g., drinking water ingestion rate of 2 L/day, bodyweight of 70 kg, exposure duration of 30 years). As shown in **Table 9**, using the recommended RfD of 0.01 mg/kg-day derived by ToxStrategies based on the HLS (2001) study, the drinking water screening level was determined to be 365 ppb. It is important to note that the exposure scenario and assumptions used to develop these drinking water screening values are consistent with that defined in the ADEC cleanup levels guidance (ADEC, 2008). Therefore, if groundwater cleanup levels were calculated using the ADEC (2008) approach and recommended RfD of 0.01 mg/kg-day, a cleanup level of 365 ppb would be calculated (i.e., the same value as the obtained using the U.S. EPA [2009b] RSL approach). In addition, the exposure scenario used by USEPA to develop drinking water RSLs is consistent with that used by USEPA to develop Maximum Contaminant Level Goals (MCLGs) for drinking water. An MCLG is the maximum level of a contaminant in drinking water at which no known or anticipated adverse effect on the health of persons would occur, and which allows an adequate margin of safety. MCLGs are non-enforceable public health goals. Since MCLGs consider only public health and not the limits of detection and treatment technology, sometimes they are set at a level that water systems cannot meet. When determining an MCLG, EPA considers the risk to sensitive subpopulations (infants, children, the elderly, and those with compromised immune systems) of experiencing a variety of adverse health effects. While the exposure scenario used by USEPA to develop the MCLG does not explicitly evaluate a child, USEPA conservatively assumes that the uncertainty factors inherent in the development of the underlying chronic RfD is adequate to address intraspecies differences

in sensitivity such as sensitive subgroups like children. In addition, because the chronic oral RfD is designed to be protective of exposures over a lifetime, it is overly conservative to use such a value in an assessment of less than lifetime exposures. As such, the drinking water screening value of 365 ppb derived by ToxStrategies is believed to be protective of both children and adults. Finally, it is also important to note that all of the values outlined in **Table 9** are conservative "screening values" that are designed to ensure adequate protection of public health. These "screening values" are not set at actual effects levels but are set well below levels where effects were observed in animals.

Table 9
Tap Water Screening Values Recommended for Sulfolane

	Description	Screening Level	Toxicity Factor Basis	Key Exposure Assumptions
Chronic Screening Levels				
	Screening level, drinking, chronic	365 ppb	Chronic RfD of 0.01 mg/kg-d[a]	Ingestion; adult
	Screening level, showering, chronic	105,000 ppb	Chronic RfC of 0.02 mg/m^3[b]	Showering scenario (Appendix B)
Acute Screening Level				
	Screening level, showering, acute	3,500,000 ppb	Acute RfC of 0.67 mg/m^3[b]	Showering scenario (Appendix B)

[a]Based on HLS (2001).
[b]Based on Andersen et al. (1977)

4.1.2 Showering

As discussed in Section 1, sulfolane is considered non-volatile. Documentation accompanying the RSL tables indicates that in order to be considered volatile (and thus be considered for tap water inhalation), chemicals must have a Henry's Law constant of 1 x 10^{-5} atmosphere-cubic meter per mole (atm-m^3/mol) or greater *and* a molecular weight less than 200 grams per mole (g/mol). While sulfolane's molecular weight is less than 200 g/mol (120 g/mol), the Henry's Law constant (reported as 1.2 x 10^{-8} atm-m^3/mol) is below the 1 x 10^{-5} atm-m^3/mol cutoff for volatility. Sulfolane is therefore considered non-volatile for the purposes of the RSL calculations and is evaluated only for ingestion exposure.

However, because the Anderson et al (1977) study demonstrated inhalation toxicity in animals exposed to sulfolane in an aerosol form, we determined that it would be important to evaluate potential human health risks associated with activities that could potentially result in the generation of mists or aerosols such as showering. As such, ToxStrategies determined the water concentrations necessary to result in air concentrations equal to the chronic and acute RfCs identified in **Table 8**. The tap water screening levels that are protective of potential inhalation exposures to sulfolane, a non-volatile compound, in a mist

or aerosol form while showering (105,000 ppb for chronic exposures over a lifetime; 3,500,000 ppb for acute exposures, see Appendix B) are much higher than the drinking water screening level (**Table 9**). The shower model results in Appendix B show that the concentration in water would have to be on the order of 105,000 ppb (or 105 mg/L) to result in an air concentration that is equal to the chronic RfC; or on the order of 3,500,000 ppb (3.5 g/L) to result in an air concentration that is equal to the acute RfC. This indicates the inhalation pathway is not significant for sulfolane, and, as such it is acceptable to develop tap water values that are based solely on drinking water ingestion.

4.2 Tap Water Screening Values Derived by Others

Several government agencies have developed acceptable levels of sulfolane in groundwater/drinking water based on sulfolane toxicity factors derived or approved by these agencies. These groundwater/drinking water values are designed to be protective for persons drinking the water.

While the U.S. EPA has not established a federal maximum contaminant level (MCL) for sulfolane, BCMWLA (2003) has established interim guideline levels of 270 ppb (based on a child) and 460 ppb (based on an adult). Additionally, while there is no Canadian drinking water quality guideline value available, CCME (2006) has established a source guidance value for groundwater of 90 ppb. Both the BCMWLA and CCME drinking water guidelines are based on the exact same toxicity study and TDI (the 13-week rat drinking water study and NOAEL of 25,000 ppb). The CCME (2006) guidance value is lower, as CCME assumed that only 20% of a person's exposure to sulfolane comes from drinking water. As already discussed in Section 3.3, the TDI used by both CCME and BCMWLA to develop their drinking water guidelines is based on the default NOAEL approach and, as such, these values do not reflect best-practices in quantitative dose-response modeling in risk assessment.

While the Alaska Department of Environmental Conservation (ADEC) has not determined official water quality criteria for sulfolane, ADEC previously developed a groundwater cleanup standard for sulfolane of 350 ppb (ADEC, 2006). This 350 ppb value assumes an average daily drinking water amount (between a child and adult) and an average body weight (between a child and adult) and is based on the same toxicity study and TDI as are the BCMWLA and CCME values (the 13-week rat drinking water study and NOAEL of 25,000 ppb), and, as such, this value does not reflect best-practices in risk assessment.

At the request of ADEC, ATSDR developed a public health action level for sulfolane in drinking water, which is intended for use as a non-regulatory screening level. The ATSDR developed three public health action levels ranging from 25 to 87.5 ppb based on three different exposure assumptions. All three action levels are based the no effect level of 0.25 mg/kg identified in by Zhu et al. (1987). ATSDR used these data to establish a NOAEL of 0.25 mg/kg/day and applied UFs totaling 100. However, as discussed above in Section 3.3, this analysis has a number of shortcomings and does not reflect best practices in risk assessment. Nevertheless, using an oral toxicity factor 0.0025 mg/kg/day, ATSDR calculated the following public health action levels in water (ATSDR, 2010):

- 25 ppb for infant scenario (based on 1 L/day ingestion rate, 10 kg body weight)
- 40 ppb for child scenario (based on 1 L/day ingestion rate, 16 kg body weight)
- 87.5 ppb for adult scenario (based on 2 L/day ingestion rate, 70 kg body weight)

While these screening values developed by ATSDR are health protective, our more refined analysis, applying benchmark dose modeling to a new data set not available at the time of ATSDR's initial assessment, indicates that these ATSDR values are overly conservative.

5.0 Conclusion

In conclusion, the tap water screening values derived by ToxStrategies are believed to be the most scientifically defensible values derived to date. The toxicity factor for the drinking water ingestion pathway is based on a subchronic GLP drinking water study and application of state-of-the-science BMD modeling. As described in Section 4.1.1, the lowest (most protective) chronic oral RfD derived by ToxStrategies (0.01 mg/kg-d) was selected for use in development of a drinking water screening level for sulfolane. The approaches used to derive this value are consistent with current U.S. EPA practices. For the RfD derivation, several endpoints were modeled using U.S. EPA's BMD software for empirical curve fitting. Among the models providing the best fits, the one providing the lowest BMDL was chosen to derive the RfD as a matter of conservatism. This value was then adjusted to a human equivalent dose to account for species differences in sensitivity due primarily to toxicokinetics. As indicated in **Table 9**, the drinking water screening level determined based on this chronic oral RfD and the above-summarized methodologies is 365 ppb. As such, concentrations below 365 ppb are not expected to pose a health risk to area residents and area residents should be able to use the groundwater and drinking water for any purpose, (e.g., drinking, showering/bathing, dishwashing, clothes washing, without experiencing any adverse health effects).

Additionally, it is important to note that even concentrations over the screening level of 365 ppb may not pose a health risk because of the manner in which this value was set. More specifically, as discussed above, the chronic oral RfD is not set at effect levels in animals or equivalent effect levels in humans, but rather, in the case of sulfolane, reflects the application of multiple uncertainty factors to the lower confidence limit of the BMD to ensure adequate protection of public health, including protection of sensitive individuals. Additionally, the exposure assumptions used to develop the tap water screening criteria are based on high-end (rather than typical) contact rates, again adding a level of protection. As such, if concentrations exceeding 365 ppb were identified in water that is used for drinking water purposes, the data would need to be closely examined to determine the likelihood that such concentrations might pose a risk to human health.

Finally, while the screening levels recently calculated by the ATSDR are no doubt health protective, our more refined analysis, applying benchmark dose modeling to a new data set not available at the time of ATSDR's initial assessment, indicates that the ATSDR values are overly conservative. Establishing regulatory guidelines at unnecessarily low levels is not always in the best interest of the public as this often results in undue fear and anxiety. As such, the goal should be to use the best available science and best risk assessment practices to establish regulatory guidelines that are protective of human health. We believe that our drinking water screening value of 365 ppb accomplishes this goal.

6.0 References

Alaska Department of Environmental Conservation (ADEC). 2008. Cleanup Levels Guidance. Division of Spill Prevention and Response, Contaminated Sites Program. June 9.

ADEC. 2006. Memorandum from D. Bauer, Environmental Engineer Associate to A. Lasater, Flint Hills Resources, Re: Approval of Corrective Action Plan. January 24.

Agency for Toxic Substances and Disease Registry (ATSDR). 2010. Health Consultation – Sulfolane. Division of Toxicology and Environmental Medicine, Prevention, Response and Medical Support Branch, Emergency Response Team. February 3.

Allen, B., Gentry, R., Shipp, A. and Van Landingham, C. (1998). Calculation of benchmark doses for reproductive and developmental toxicity observed after exposure to isopropanol. Regul Toxicol Pharmacol 28, 38-44.

Andersen, M.E., R.A. Jones, L. Kurlansik, R.G. Mehl, and L.J. Jenkins Jr. 1976. Sulfolane-Induced Convulsions in Rodents. Res. Comm. Chem. Pathol. Pharmacol. 15:571-580.

Andersen, M.E., R.A. Jones, R.G. Mehl, T.A. Hill, L. Kurlansik, and L.J. Jenkins Jr. 1977. The Inhalation Toxicity of Sulfolane (Tetrahydrothiophene-1,1-dioxide). Toxicol. App. Pharmacol. 40:463-472.

British Columbia Ministry of Water, Land and Air Protection (BCMWLA). 2003. Water Quality Guidelines for Sulfolane. Prepared by Komex International Ltd. August 6.

Brown, V.K.H., L.W. Ferrigan, and D.E. Stevenson. 1966. Acute Toxicity and Skin Irritant Properties of Sulfolane. Brit. J. Industr. Med. 23:302-304.

Canadian Association of Petroleum Producers (CAPP). 2001. Soil and Water Quality Guidelines for Sulfolane and Diisopropanolamine (DIPA): Environmental and Human Health. Unpublished report prepared by Komex International Ltd. File No. C50560000.

Canadian Council of Ministers of the Environment (CCME). 2006a. Canadian Environmental Quality Guidelines for Sulfolane: Water and Soil, Scientific Supporting Document.

CCME. 2006b. Canadian Soil Quality Guidelines for the Protection of Environmental and Human Health: Sulfolane. Canadian Environmental Quality Guidelines.

Crump, K. S. (1984). A new method for determining allowable daily intakes. Fundam Appl Toxicol 4, 854-71.

European Commission, European Chemicals Bureau. 2000. International Uniform Chemical Information Database (IUCLID) Dataset, Substance ID: 126-33-0. February 18. http://ecb.jrc.ec.europa.eu/esis/

Gaylor, D., Ryan, L., Krewski, D. and Zhu, Y. (1998). Procedures for calculating benchmark doses for health risk assessment. Regul Toxicol Pharmacol 28, 150-64.

Gordon, C. J. (2005). Temperature and Toxicology. CRC Press, Boca Raton.

Hard, G. C., Rodgers, I. S., Baetcke, K. P., Richards, W. L., McGaughy, R. E. and Valcovic, L. R. (1993). Hazard evaluation of chemicals that cause accumulation of alpha 2u-globulin, hyaline droplet nephropathy, and tubule neoplasia in the kidneys of male rats. Environ Health Perspect 99, 313-49.

Huntingdon Life Sciences (HLS). 2001. Sulfolane Toxicity Study by Oral Administration via the Drinking Water Pathway to CD Rats for 13 Weeks. Huntingdon Life Sciences Ltd. Huntingdon, England. As cited in BCMWLA, 2003.

ICH (2005). ICH Harmonised Tripartite Guideline, Impurities: Guideline for Residual Solvents Q3C (R3).

Jonckheere, A. R. (1954). A Distribution-Free K-Sample Test Against Ordered Alternatives. Biometrika 41, 133-145.

Leisenring, W. and Ryan, L. (1992). Statistical properties of the NOAEL. Regul Toxicol Pharmacol 15, 161-71.

Mohler, F.S. and C.J. Gordon. 1988. Thermoregulatory Responses of the Rabbit to Subcutaneous Injection of Sulfolane. Arch. Toxicol. 62:216-219.

National Cancer Institute. 1978. Bioassay of 3-Sulfolene for Possible Carcinogenicity. Carcinogenesis: Technical Report Series. No. 102. National Cancer Institute. Obtained from the Office of Toxic Substances, U.S. Environmental Protection Agency. Washington, D.C.

OECD (2004). SIDS Initial Assessment Report for: Tetrahydrothiophene-1,1-dioxide (CAS, No. 126-33-0).
http://www.chem.unep.ch/irptc/sids/OECDSIDS/indexcasnumb.htm

Roberts, J. J. and Warwick, G. P. (1961). The mode of action of alkylating agents. III. The formation of 3-hydroxytetrahydrothiophene-1: 1-dioxide from 1 :4-dimethylsulphonyloxy-butyrate (myleran), S-β-L-alanyltetrahydrothiopheniummesylate, tetrahydrothiophene and tetrahydrothiophene-1 : 1-dioxide in the rat, rabbit and mouse. Biochem Pharmacol 6, 217-227.

Suckow (2006). The Laboratory Rat. Elsevier Academic Press, Burlington, MA.

U.S. Environmental Protection Agency (U.S. EPA). 2009a. Integrated Risk Information System Glossary. Available at http://www.epa.gov/IRIS/help_gloss.htm

U.S. EPA. 2009b. Regional Screening Levels for Chemical Contaminants at Superfund Sites. Available at http://www.epa.gov/reg3hwmd/risk/human/rb-concentration_table/index.htm

U.S.EPA (2006). Harmonization in Interspecies Extrapolation: Use of BW 3/4 as Default Method in Derivation of the Oral RfD (External Review Draft).

U.S. EPA. 2002a. A Review of the Reference Dose and Reference Concentration Processes. EPA/630/P-02/002F. Washington, DC: Risk Assessment Forum. Available at http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=55365

U.S.EPA (2002b). Toxicological Review of Benzene (Noncancer Effects) (Cas No. 71-43-2).

U.S. EPA. 1997a. Exposure Factors Handbook, Office of Research and Development, Washington, DC. Available at http://www.epa.gov/ncea/efh/pdfs/efh-front-gloss.pdf. Last accessed November 8, 2009

U.S. EPA 1997b. Supplemental Background Document; Non-Groundwater Pathway Risk Assessment; Petroleum Process Waste Listing Determination. EPA Contract No. 68-W6-0053; RTI Project No. 92U-6720-001. http://www.epa.gov/waste/hazard/wastetypes/wasteid/petroref/contents.pdf. Last accessed November 8, 2009.

U.S. EPA. 1996. Exposure Factors Handbook (Draft), Office of Research and Development, Washington, DC. April. NTIS PB97-117675.

U.S.EPA (1991). Report of the EPA Peer Review Workshop on Alpha2u-Globulin: Association with Renal Toxicity and Neoplasia in the Male Rat.

U.S.EPA (1988). Recommendations for and Documentation of Biological Values for Use in Risk Assessment. Office of Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency. EPA/600/6-87/008

Zhu, Z.H., M.L. Sun, Z.S. Li, Z.C. Yang, T.B. Zhang, Z.C. Heng, B.L. Xiao, Q.Y. Li, Q.Y. Peng, and Y.H. Dong. 1987. An Investigation of the Maximum Allowable Concentration of Sulfolane in Surface Water (Chinese). J. West China Univ. Med. Soc. [Hua Hsi I Ko Ta Hsueh Hsueh Pao]. 18:376-380.

Zhu, Z.H., N. Gao, J.T. Guo, M.L. Sun, D.S. Wu, Z.C. Yang, Z.M. Li, and Y.D. Lei. 1988. Studies on the Toxicokinetics of 3H-Sulfolane in Rat after Oral Administration (Chinese). J. West China Univ. Med. Soc. [Hua Hsi I Ko Ta Hsueh Hsueh Pao]. 19:61-64.

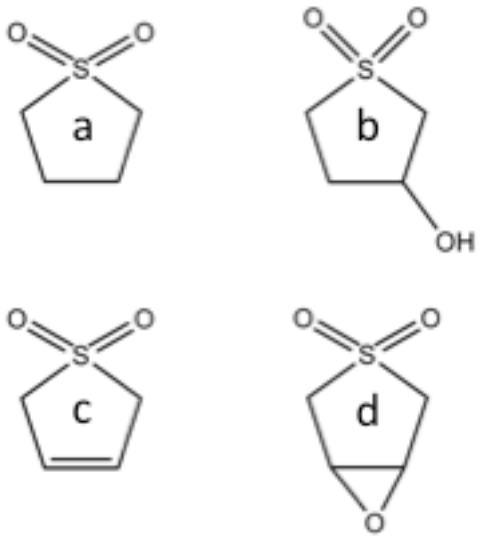
Appendix A: Summary of Toxicological Studies and Chemical Structures of Sulfolane and Related Compounds

Table A1: Summary of Toxicological Studies on Sulfolane			
Species	**Treatment**	**Effects**	**Reference**
Acute Toxicity			
rat	oral gavage	LD50 = 2,100 mg/kg	Brown et al. (1966)
mouse	oral gavage	LD50 = 1,900-2,500 mg/kg	Brown et al. (1966)
rat	oral gavage	LD50 = 1,846 mg/kg	Andersen et al. (1976)
guinea pigs	oral gavage	LD50 = 1,815 mg/kg	Andersen et al. (1976)
rat	oral gavage	LD50 = 2,343 mg/kg	Zhu et al. (1987)
mouse	oral gavage	LD50 = 2,504 mg/kg	Zhu et al. (1987)
guinea pigs	oral gavage	LD50 = 1,445 mg/kg	Zhu et al. (1987)
rat	oral gavage	LD50 = 2,006 mg/kg (males);2,130 mg/kg (females)	OECD (2004)
rat	i.p.	LD50 = 1,598 mg/kg	Andersen et al. (1976)
mouse	i.p.	LD50 = 1,331 mg/kg	Andersen et al. (1976)
rat	s.c.	LD50 = 1,606 mg/kg	Andersen et al. (1976)
mouse	s.c.	LD50 = 1,360 mg/kg	Andersen et al. (1976)
rat	i.v.	LD50 = 1,094 mg/kg	Andersen et al. (1976)
mouse	i.v.	LD50 = 632 mg/kg	Andersen et al. (1976)
rat	inhalation	LC50 = 4,700 mg/m^3	Andersen et al. (1977)
rat	inhalation, 3,600 mg/m^3, 18 hr	decreased WBC (animals found in extremis)	Andersen et al. (1977)
monkey	inhalation, 4,850 mg/m^3, 19 hr	decreased WBC, hematocrit, hemoglobin; pulmonary hemorrhage (animals convulsed)	Andersen et al. (1977)
mouse, rat, rabbit	multiple	changes in thermoregulation	Gordon (2005)
Irritation and Sensitization			
rat	dermal	no effect, LD50 > 3,800 mg/kg	Brown et al. (1966)
rat	dermal	LD50 > 2,000 mg/kg	ECECB (2000), ref #38
rabbit	dermal	LD50 12,600 mg/kg	OECD (2004), ref #9
rabbit	dermal	no irritation, no skin damage	Brown et al. (1966)
guinea pigs	dermal	no irritation, no skin damage	Brown et al. (1966)

guinea pigs	dermal, intradermal	no sensitization	Brown et al. (1966)
guinea pigs	dermal, intradermal	no sensitization	OECD (2004) Ref# 12
rabbit	instillation to eye	temporary mild irritation	Brown et al. (1966)
rabbit	instillation to eye	moderately irritating	ECECB (2000) Ref# 51
Repeated Dose Toxicity			
rat	unclear (likely oral gavage), 90 days; 55.6, 167, 500 mg/kg	decreases in serum alkaline phosphatase aminotransferase at 500 mg/kg	Zhu et al. (1987)
guinea pig	unclear (likely oral gavage), 90 days; 55.6, 167, 500 mg/kg	decreases in serum alkaline phosphatase and WBC at 55.6 mg/kg	Zhu et al. (1987)
guinea pig	unclear (likely oral gavage), 90 days; 0.25, 2.5, 25, 250 mg/kg	changes in white pulp of spleen, variable changes in serum chemistry, reduced bone marrow cell counts	Zhu et al. (1987)
guinea pig	unclear (likely oral gavage), 6 months; 0.25, 2.5, 25, 250 mg/kg	histopathological changes in the liver (fatty liver) and spleen (white pulp), reduced bone marrow cell counts	Zhu et al. (1987)
rat	drinking water, 90 days; 25, 100, 400, 1600 mg/L	male NOAEL of 8.8 mg/kg/day for hydrocarbon nephropathy; female NOEL of 2.9 mg/kg/day for reduced white blood cells	HLS (2001)
rat	50 mg/kg 6 times per week for 4 months	No adverse effects on bodyweight, behavioral or blood (details not provided)	ECECB (2000) Ref# 56
rat	gavage 28 days with 14 day post exposure period; at 60, 200, and 700 mg/kg	female NOAEL of 200 mg/kg/day for reduced bodyweight and food consumption; male NOAEL of 60 mg/kg based on changes in kidney (hydrocarbon nephropathy)	OECD (2004) Ref# 24
rat	inhalation, 495 mg/m^3, 27 days	fatty liver, lung inflammation, decreased WBC (non-significant)	Andersen et al. (1977)
guinea pig	inhalation, 495 mg/m^3, 27 days	no significant hematological, blood biochemical or bodyweight effects	Andersen et al. (1977)
dog	inhalation, 495 mg/m^3, 27 days	no significant hematological, blood biochemical or bodyweight effects	Andersen et al. (1977)
monkey	inhalation, 495 mg/m^3, 27 days	fatty liver, decreased WBC (non-significant), death	Andersen et al. (1977)
guinea pig	inhalation, 200 mg/m^3, 23 hr/day for 90 days	fatty liver, reduced WBC at 20, 30, and 90 days	Andersen et al. (1977)
dog	inhalation, 200 mg/m^3, 23 hr/day for 90 days	CNS effects (seizure) lung inflammation, death	Andersen et al. (1977)
monkey	inhalation, 200 mg/m^3, 23 hr/day for 90 days	death and morbidity (some animals had parasites)	Andersen et al. (1977)
guinea pig	inhalation, 159 mg/m^3, 23 hr/day for 85 days	no changes in WBC, hematocrit or hemoglobin	Andersen et al. (1977)

rat, guinea pig, dog, monkey	inhalation, 20 mg/m^3, 23 hr/day for 90 days	no adverse effects reported	Andersen et al. (1977)
Reproductive and Developmental Toxicity			
rat	gavage 41-50 days at 60, 200, and 700 mg/kg	male NOAEL = 700 mg/kg for reproductive performance; female NOAEL of 200 mg/kg for reduction in estrus cycles; pup NOAEL of 60 mg/kg for reduced birth index and number of pups alive at day o and 4 of lactation	OECD (2004)
mouse	unclear (likely oral gavage), gestation days 6-15; 93, 280, 840 mg/kg; sacrificed on 18th day of gestation	no abnormalities or resorptions at 280 mg/kg	Zhu et al. (1987)
rat	s.c. gestation days 6-15; 25, 100, 400 mg/kg	reported NOEL for teratogenicity at 400 mg/kg	ICH (2005)
Carcinogenicity			
rat	3-sulfolene: 197 and 372 mg/kg for males; 120 and 240 mg/kg female; 78 wk exposure with 33 wk observation period	no evidence of carcinogenicity; last male died at wk 60 in high dose group (i.e. no observation period in these animals)	NCI (1978)
mouse	3-sulfolene: 311 and 622 mg/kg for males; 384 and 768 mg/kg female with 13 wk observation period	no evidence of carcinogenicity	NCI (1978)

Table A2: Summary of Genotoxicity Studies on Sulfolane			
In vivo/In vitro	**Test System**	**Effects**	**Reference**
in vivo, mouse	single oral dose; 62.5, 125, 250, 500, 1000 mg/kg	no increase in micronucleus rate	Zhu et al. (1987)
in vitro	human lymphocytes; 0.01, 0.1, 1, 10 mg/mL	no sister chromatid exchange observed	Zhu et al. (1987)
in vitro	Salmonella strains (TA1535, TA1537, TA1538, TA98, and TA100) with and with activation	negative	Zhu et al. (1987)
in vitro, GLP	Salmonella strains (TA1535, TA1537, TA1538, TA98, and TA100) with and with activation	negative	ECECB (2000), ref #60
in vitro, GLP	E. coli reverse mutation assay with and without activation (WP2, WP2 uvrA);	negative	ECECB (2000), ref #60
in vitro	S. cerevisiae with and without activation	negative	ECECB (2000), ref #60
in vitro	RL4 cells without activation	negative	ECECB (2000), ref #60
in vitro	Chinese hamster V79 cells	negative	ECECB (2000), ref #61
in vitro, GLP, OECD 471, 472	Salmonella strains (TA1535, TA1537, TA98, and TA100) with and with activation	negative	OECD (2004), ref #25
in vitro, GLP, OECD 471, 472	E. coli with and with activation	negative	OECD (2004), ref #25
in vitro, GLP	Chinese hamster CHL/IU cells	negative	OECD (2004), Ref# 26
in vitro	Salmonella strains (TA1535, TA1537, TA1538, TA98, and TA100) with and with activation	negative	OECD (2004), ref #31
in vitro	Chinese hamster ovary cells	negative	OECD (2004), Ref# 34
in vitro	mouse lymphoma assay	positive with and without activation; IUCLID file indicates no dose-response relationship	OECD (2004), ref #32



Chemical Structures: a) sulfolane, b) 3-hydroxysulfolane (major metabolite of sulfolane), c) sulfolene (noncarcinogenic), d) 3,4-epoxysulfolane (carcinogenic)

Appendix B: Estimating Air Concentrations Associated with Sulfolane, a Non-Volatile, in Showers

Transfer of chemical contaminants from potable household water to indoor air has been well documented in the literature (Gunderson and Witham, 1998; Finley et al., 1996; Xu and Weisel, 2003; Keating and McKone, 1993). Some studies have estimated the inhalation dose from indoor potable water sources at around 50 to 75% of the daily ingested dose from 2 liters of tap water for volatile compounds (McKone, 1987; Wilkes et al., 1992). The presence of chemical contaminants in the shower water has been of particular interest because of the favorable conditions for the transfer of chemicals to air, and the high volume of water typically used. Non-volatile chemicals may be introduced to the air by entrainment of water particles in air as aerosols, whereas volatile chemicals may be introduced as aerosols or as vapor emissions. The purpose of this discussion is to document a methodology for estimating the airborne concentration of a non-volatile compound such as sulfalone in a shower scenario. This provides a means of estimating a level in water that is equivalent to the acute and chronic RfCs derived in Section 3.2.2.

The rationale for our shower model can be expressed in mathematical terms, as demonstrated below in Equation 1. The A term in this equation is a constant that is derived from empirical data. In this case, the empirical data is gathered from Keating and McKone (1993), in which the authors specifically measure the range of aerosol droplet sizes and count of aerosols generated from three different showers. This same study and data is used by CalEPA in their derivation of a public health goal (PHG) for hexavalent chromium in drinking water (CalEPA, 2009).

Eq. 1 C_{air} (ug/m^3) = C_{water} (mg/L) * A (ug-L/mg-m^3)

In their study, Keating and McKone conducted shower simulations in an exposure chamber that measured 1.08 m by 0.75 m by 1.3 meters (l, w, h). The shower nozzle was located approximately 120 cm from the floor of the chamber. Each simulation lasted 1 hour, during which the shower was run for periods up to 10 min during which time air and water samples were taken. After water flow stopped, the air was sampled for the remainder of the hour. Aerosol concentrations were measured for the first 15 min of each simulation. The air flow rate in the chamber was 65 L/min, the temperature of the water ranged from 40 to 50C.

Of the three shower nozzles that were used in the study, two were designed for commercial use, and one was a water-saving nozzle designed for use in a home shower (Teledyne Water Pic, Fort Collins, CO). The focus of this discussion is on the nozzle designed for home use. This nozzle was rated for a droplet size of 1000 um, and a flow rate of 6 L/min. Aerosols were measured using Army Insecticide Measurement System (AIMS) Droplet Counter (KLD Labs, Huntington Station, NY). This system is a hot wire anemometer probe

which is cooled when droplets come in contact with a thin platinum wire; reducing the probe's electrical resistance. The resultant signal is dependent on droplet size. The probe was positioned at the same height as the shower nozzle and aerosol concentration was measured in the chamber for the first 15 min of the 1 hour simulation.

Keating and McKone (1993) report results for the home-use nozzle; the aerosol concentration in the shower chamber was 1022 aerosols per cm^3, and the median aerosol droplet diameter was 7.1 um. These two pieces of data may be used to calculate the total volume concentration of aerosol water in the chamber during showering. First, the diameter is converted to a volume, as demonstrated in Equation 2.

Eq. 2 $V_{aerosol}$ = 4/3 * pi * (aerosol radius)^3
= 4/3 * 3.14159 * (7.1 um/2)^3
= 187 um^3

Next, the volume of the median aerosol is multiplied by the number of aerosols to calculate the volume of aerosol water per unit volume of shower air, as demonstrated below in Equation 3. Up to this point, these calculations are exactly reflected in the CalEPA derivation of the PHG for hexavalent chromium (CalEPA, 2009).

Eq. 3 $C_{aerosol, shower}$ = $V_{aerosol}$ * (aerosols/cm^3)
= 187 um^3/aerosol * 1022 aerosols/cm^3
= 191,500 $um^3_{aerosol}$ /$cm^3_{shower\ air}$

The aerosol volume is then converted from um^3 to cm^3 according to Equation 4 below.

Eq. 4 $C_{aerosol, shower}$ (cm^3/cm^3) = $C_{aerosol, shower}$ (um^3/cm^3) *
(m^3/1E18 um^3) * (1E6 cm^3/m^3)
= 1.92E-7 cm^3/cm^3

We then convert this concentration (cm3/cm3) to the units of our constant, A (ug-L/mg-m^3) by first multiplying by unity (mg/mg), as demonstrated below in Equation 5.

Eq. 5 1.92E-7 cm^3/cm^3 * 1 mg/mg = 1.92E-7 mg-cm^3/mg-cm^3

Next, we apply a few units conversions, as demonstrated in Equation 6.

Eq. 6 A = 1.92E-7 mg-cm^3/mg-cm^3 * 1E3 ug/mg * L/1E3 cm^3 * 1E6 cm^3/m^3
= 0.192 ug-L/mg-m^3

The A calculated in equation 6 above may be used to convert water concentrations (in mg/L) to shower air concentrations (in ug/m^3) for non-volatiles. This is demonstrated below in the reapplication of Equation 1 for a water concentration of 100 ppb (ug/L).

Eq. 1 C_{air} (ug/m^3) = C_{water} (mg/L) * A (ug-L/mg-m^3)
= 100 ug/L * (mg/1000 ug) * 0.192 ug-L/mg-m^3
= 0.0192 ug/m^3
= 1.92E-5 mg/m^3

With this model, one can estimate the concentration in water that is associated with health protective benchmarks in air such as the acute and chronic RfCs derived in Section 3.2.2. Inputting the acute RfC of 0.67 mg/m3 into the model yields a concentration in water of 3,500,000 ppb. This means that the concentration of sulfalone in water would have to be on the order of 3,500,000 ug/L (3.5 g/L) to result in an air concentration that is equal to the acute RfC. Any concentrations below this would not pose an acute health threat while showering. Likewise, inputting the chronic RfC of 0.02 mg/m3 into the model yields a concentration in water of 105,000 ppb. This means that the concentration of sulfalone in water would have to be on the order of 105,000 ug/L (or 105 mg/L) to result in an air concentration that is equal to the chronic RfC. Any concentrations below this would not pose a chronic health threat if exposed every day throughout one's lifetime.

References

California Environmental Protection Agency (CalEPA). 2009. *Public Health Goal for Hexavalent Chromium in Drinking Water (Draft)*. Pesticide and Environmental Toxicology Branch, OEHHA. http://oehha.ca.gov/water/phg/chr6draft082009.html. Last accessed, November 16, 2009.

Finley, B.L., B.D. Kerger, D.G. Dodge, S.M. Meyers, R.O. Richter, and D.J. Paustenbach. 1996. *Assessment of airborne hexavalent chromium in the home following use of contaminated tapwater*. J. Exposure Anal. Environ. Epidemiol. 6(2):229–245.

Gunderson, E.C., and C.L. Witham. 1988. *Determination of inhalable nonvolatile salts with a shower*. SRI International, SRI Project No. PYC-6631, Menlo Park, CA.

Keating, G., and McKone, T. 1993. *Measurements and Evaluation of the Water to Air Transfer and Air Concentration for Trichloroethylene in a Shower Chamber*. Modeling of Indoor Air Quality and Exposure. ASTM STP 1205, NirenL. Nagda, Ed., American Society for Testing and Materials, Philadelphia, 1993. Pp. 14-24.

McKone, T. 1987. *Human Exposure to Volatile Organic Compounds in Household Tap Water: The Indoor Inhalation Pathway*. Environmental Science and Technology. Vol. 21, 1987, p. 1194.

Wilkes, C., M. Small, J. Andelman, N. Giardino, and J. Marshall. 1992. *Inhalation exposure model for volatile chemical from indoor uses of water*. Atmospheric Environment 26a:2227.

Xu, X., and C.W. Weisel. 2003. *Inhalation exposure to haloacetic acids and haloketones during showering*. Environ. Sci. Technol. 37:569–576.

Appendix C: Benchmark Dose Modeling of HLS (2001)

As described in the body of the report, effects on reduced WBC and lymphocytes were modeled in the U.S. EPA BMDS after log transformation of dose. The linear, power, and exponential models were all found to provide reasonable fits to the data. In fact, the power model provided the same results as the linear model. Among the submodels that are run simultaneously with the exponential model, submodels 2 and 4 gave acceptable fits to the data (**Table C1**). The output from the BMDS for the continuous models in **Table C1** are included at the end of this discussion. As already described above, the results of the linear model were chosen as the POD because the linear model provided the best fit overall and is also the most parsimonious of the models tested.

Another approach to modeling continuous data is to dichotomize the data and model the incidence of adverse effects (U.S. EPA, 2000). This approach was explored because there is uncertainty as to whether the observed changes in white blood cell counts truly represent an adverse health effect. Typically, reference ranges for hematological values are considered normal when they fall within ± 2 standard deviations from the mean value (Sucklow et al., 2006). As such, historical control mean and standard deviation values were also used to set cutoffs for scoring the incidence of low cell count in individual animals in the HLS sulfolane study. Therefore, two cutoff values were chosen for the WBC and lymphocytes datasets: (-) 2 standard deviations from the mean using the historical HLS data, and (-) 2 standard deviations from the mean in the HLS concurrent controls. Using (-) 2 standard deviations from the concurrent and historical HLS datasets resulted in identical incidences for reduced WBC and lymphocytes, which is not unexpected given that lymphocytes comprise the vast majority of white blood cells. The $BMDL_{10}$ for reduction in white blood cells in female rats exposed to sulfolane in drinking water was determined to be 21.8 mg/kg/day (**Table C1**).

Although the dichotomization approach takes into account the biological relevance of the reduced WBC count through scoring individual animals as having abnormally low cell counts, the small sample size makes this approach less robust due to the loss of information and statistical power after converting the data to incidence. Among the continuous models that fit the WBC data, submodels 2 and 4 gave slightly higher p-values and slightly lower[6] Akaike's Information Criterion (AIC) values than the linear model, but the differences were not enough to clearly establish which model has the better fit (**Table C1**). In contrast, the scaled residuals closest to the BMD were about 2-fold lower in the linear model than the exponential models. Furthermore, in evaluating the EPA BMD modeling results of benzene on lymphocytes using this modeling approach, the EPA stated that the "the linear model was selected because it is the most parsimonious" (U.S. EPA, 2002b). Given that the reduction in WBC counts were not clearly adverse effects, the BMDL

[6] lower AIC values indicate a better fit of the model to the data.

values from the parsimonious linear models were selected as the POD as was done in the assessment for benzene (U.S. EPA 2002b).

Table C1. Summary of Benchmark Modeling Results

Model Parameter	BMD Modeling Results			
	Continuous			Dichotomized[3]
WBC				
Model	**Linear[1]**	**Exponential**		**LogLogistic**
Submodel		**M2**	**M4**	
p-values	0.1677	0.1755	0.1755	0.54
scaled residual	0.168	0.3819	0.3819	0.322
AIC	113.08	112.97	112.97	28.6
BMD^2, ln(dose+1)	4.22	3.81	3.81	NA
BMDL, ln(dose+1)	2.78	2.23	1.78	NA
BMD	67.03	44.15	44.15	68.9
BMDL	15.12	8.30	4.93	21.8
Lymphocytes				
Model	**Linear[1]**	**Exponential**		**LogLogistic**
Submodel		**M2**	**M4**	
p-values	0.158	0.1678	0.1678	0.54
scaled residual	0.232	0.4715	0.4715	0.322
AIC	102.61	102.46	102.46	28.6
BMD^2, ln(dose+1)	4.34	3.86	3.86	NA
BMDL, ln(dose+1)	2.83	2.19	1.68	NA
BMD	75.71	46.28	46.47	68.9
BMDL	15.95	7.94	4.37	21.8

[1] identical values were also obtained with the BMDS Power Model

[2] Because the doses were log transformed, the BMD and BMDL values reported in the BMD software output were ln(dose + 1) and were mathematically converted back to arithmetic scale for reporting BMD and BMDL

[3] Modeling not shown

WBC, Log Dose, Historical HLS SD - Exponential

```
 ================================================================
        Exponential Model. (Version: 1.61;  Date: 7/24/2009)
        Input Data File: C:\USEPA\BMDS21\Data\exp_HLS-WBC_logdose-historicalHLS-
SD_Setting.(d)
        Gnuplot Plotting File:
                                                Wed Jun 02 13:47:59 2010
 ================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function by Model:
      Model 2:     Y[dose] = a * exp{sign * b * dose}
      Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
      Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
      Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

    Note: Y[dose] is the median response for exposure = dose;
          sign = +1 for increasing trend in data;
          sign = -1 for decreasing trend.

      Model 2 is nested within Models 3 and 4.
      Model 3 is nested within Model 5.
      Model 4 is nested within Model 5.


   Dependent variable = Response
   Independent variable = Dose
   Data are assumed to be distributed: normally
   Variance Model: exp(lnalpha +rho *ln(Y[dose]))
   The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

   Total number of dose groups = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

   MLE solution provided: Exact


                                Initial Parameter Values

     Variable          Model 2           Model 3           Model 4           Model 5
     --------          -------           -------           -------           -------
      lnalpha          -4.46856          -4.46856            -4.46856          -
4.46856
          rho           3.12885           3.12885             3.12885
3.12885
            a           4.59626           4.59626               8.3685
8.3685
            b          0.111231          0.111231             0.140286
0.140286
            c                --                --               0.108502
0.108502
            d                --                 1                  --
1


                              Parameter Estimates by Model
```

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	-4.38524	-4.46381	-4.38524	-4.27557
rho	3.07751	3.12036	3.07751	3.01475
a	8.10467	7.97624	8.10467	8.00292
b	0.110789	0.114998	0.110789	0.281389
c	--	--	0	0.481718
d	--	1.10869	--	1.47486

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0	10	7.97	2.626
1.361	10	7.63	2.653
2.451	9	5.41	1.392
3.761	9	5.53	1.756
5.258	10	4.54	1.109

Estimated Values of Interest

Model	Dose	Est Mean	Est Std	Scaled Residual
2	0	8.105	2.793	-0.1525
	1.361	6.97	2.215	0.942
	2.451	6.177	1.839	-1.252
	3.761	5.343	1.471	0.3819
	5.258	4.526	1.14	0.03795
3	0	7.976	2.739	-0.007205
	1.361	7.018	2.244	0.862
	2.451	6.239	1.867	-1.332
	3.761	5.374	1.479	0.3161
	5.258	4.5	1.121	0.114
4	0	8.105	2.793	-0.1525
	1.361	6.97	2.215	0.942
	2.451	6.177	1.839	-1.252
	3.761	5.343	1.471	0.3819
	5.258	4.526	1.14	0.03795
5	0	8.003	2.711	-0.0384
	1.361	7.109	2.268	0.7268
	2.451	6.182	1.837	-1.261
	3.761	5.254	1.437	0.577
	5.258	4.553	1.159	-0.03603

Other models for which likelihoods are calculated:

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)

Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

Model R: Yij = Mu + e(i)
Var{e(ij)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-55.27087	6	122.5417
A2	-49.84968	10	119.6994
A3	-50.01255	7	114.0251
R	-65.0528	2	134.1056
2	-52.48727	4	112.9745
3	-52.45111	5	114.9022
4	-52.48727	4	112.9745
5	-52.36868	6	116.7374

Additive constant for all log-likelihoods = -44.11. This constant added to the above values gives the log-likelihood including the term that does not depend on the model parameters.

Explanation of Tests

Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A2 vs. A1)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does Model 2 fit the data? (A3 vs. 2)

Test 5a: Does Model 3 fit the data? (A3 vs 3)
Test 5b: Is Model 3 better than Model 2? (3 vs. 2)

Test 6a: Does Model 4 fit the data? (A3 vs 4)
Test 6b: Is Model 4 better than Model 2? (4 vs. 2)

Test 7a: Does Model 5 fit the data? (A3 vs 5)
Test 7b: Is Model 5 better than Model 3? (5 vs. 3)
Test 7c: Is Model 5 better than Model 4? (5 vs. 4)

Tests of Interest

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	30.41	8	0.0001791
Test 2	10.84	4	0.02839
Test 3	0.3257	3	0.9551
Test 4	4.949	3	0.1755
Test 5a	4.877	2	0.08729
Test 5b	0.07233	1	0.788
Test 6a	4.949	3	0.1755
Test 6b	0	0	N/A
Test 7a	4.712	1	0.02995
Test 7b	0.1649	1	0.6847
Test 7c	0.2372	2	0.8882

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels, it seems appropriate to model the data.

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate.

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here.

The p-value for Test 4 is greater than .1. Model 2 seems to adequately describe the data.

The p-value for Test 5a is less than .1. Model 3 may not adequately describe the data; you may want to consider another model.

The p-value for Test 5b is greater than .05. Model 3 does not seem to fit the data better than Model 2.

The p-value for Test 6a is greater than .1. Model 4 seems to adequately describe the data.

Degrees of freedom for Test 6b are less than or equal to 0. The Chi-Square test for fit is not valid.

The p-value for Test 7a is less than .1. Model 5 may not adequately describe the data; you may want to consider another model.

The p-value for Test 7b is greater than .05. Model 5 does not seem to fit the data better than Model 3.

The p-value for Test 7c is greater than .05. Model 5 does not seem to fit the data better than Model 4.

Benchmark Dose Computations:

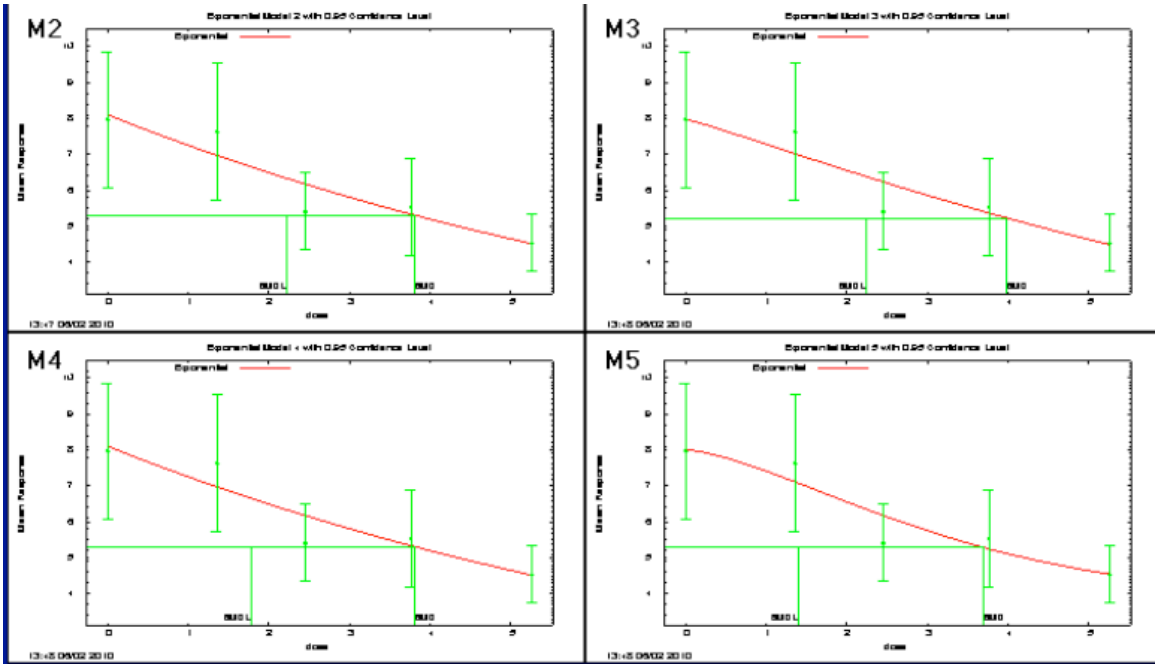
Specified Effect = 1.000000

Risk Type = Estimated standard deviations from control

Confidence Level = 0.950000

BMD and BMDL by Model

Model	BMD	BMDL
2	3.81396	2.22988
3	3.98291	2.24415
4	3.81396	1.78368
5	3.69718	1.40591


M2
M3
M4
M5

WBC, Log Dose, Historical HLS SD - Linear

```
 ======================================================================
        Polynomial Model. (Version: 2.13;  Date: 04/08/2008)
        Input Data File: C:\USEPA\BMDS21\Data\lin_HLS-WBC_logdose-historicalHLS-
SD_Setting.(d)
        Gnuplot Plotting File:  C:\USEPA\BMDS21\Data\lin_HLS-WBC_logdose-
historicalHLS-SD_Setting.plt
                                              Wed Jun 02 14:30:09 2010
 ======================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...


   Dependent variable = Response
   Independent variable = Dose
   Signs of the polynomial coefficients are not restricted
   The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

   Total number of dose groups = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                         lalpha =      1.41295
                            rho =            0
                         beta_0 =      7.97188
                         beta_1 =    -0.684223


           Asymptotic Correlation Matrix of Parameter Estimates

                lalpha          rho       beta_0       beta_1

    lalpha           1        -0.99         0.11        -0.13

       rho       -0.99            1        -0.11         0.13

    beta_0        0.11        -0.11            1        -0.91

    beta_1       -0.13         0.13        -0.91            1


                                Parameter Estimates

                                                     95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
        lalpha          -4.6222          1.92724           -8.39952           -
0.844874
            rho          3.21044           1.0586            1.13562
```

5.28525
beta_0 7.8725 0.56274 6.76955
8.97545
beta_1 -0.64517 0.137292 -0.914258 -
0.376082

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	10	7.97	7.87	2.63	2.72	0.113
1.361	10	7.63	6.99	2.65	2.25	0.893
2.451	9	5.41	6.29	1.39	1.9	-1.39
3.761	9	5.53	5.45	1.76	1.51	0.168
5.258	10	4.54	4.48	1.11	1.1	0.172

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
Model A3 uses any fixed variance parameters that
were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-55.270870	6	122.541740
A2	-49.849681	10	119.699362
A3	-50.012552	7	114.025103
fitted	-52.540846	4	113.081693
R	-65.052796	2	134.105592

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	30.4062	8	0.0001791

Test 2	10.8424	4	0.02839
Test 3	0.325741	3	0.9551
Test 4	5.05659	3	0.1677

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels It seems appropriate to model the data

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems to adequately describe the data

Benchmark Dose Computation

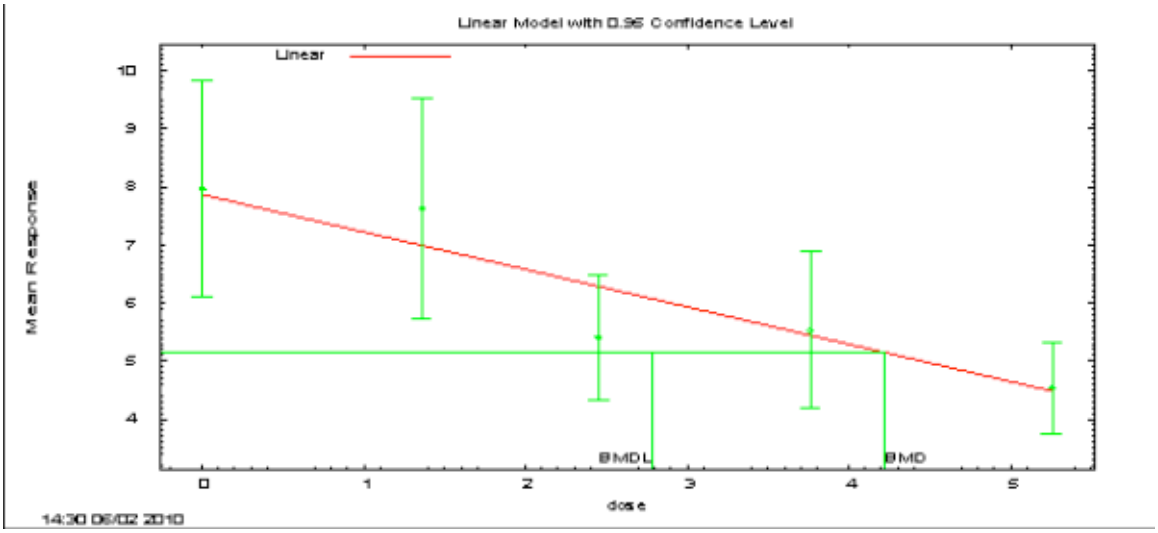
Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 4.21778

BMDL = 2.78079



Lymphocytes, Log Dose, Historical HLS SD - Exponential

```
 ================================================================
        Exponential Model. (Version: 1.61;  Date: 7/24/2009)
        Input Data File: C:\USEPA\BMDS21\Data\exp_HLS-Lymphocytes-logdose-
historicalHLS-SD_Setting.(d)
        Gnuplot Plotting File:
                                              Wed Jun 02 14:55:55 2010
 ================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function by Model:
      Model 2:     Y[dose] = a * exp{sign * b * dose}
      Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
      Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
      Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

    Note: Y[dose] is the median response for exposure = dose;
          sign = +1 for increasing trend in data;
          sign = -1 for decreasing trend.

      Model 2 is nested within Models 3 and 4.
      Model 3 is nested within Model 5.
      Model 4 is nested within Model 5.


   Dependent variable = Response
   Independent variable = Dose
   Data are assumed to be distributed: normally
   Variance Model: exp(lnalpha +rho *ln(Y[dose]))
   The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

   Total number of dose groups = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

   MLE solution provided: Exact


                                Initial Parameter Values

     Variable          Model 2           Model 3           Model 4           Model 5
     --------          -------           -------           -------           -------
      lnalpha          -3.80574          -3.80574            -3.80574          -
3.80574
          rho           2.92924           2.92924             2.92924
2.92924
            a           3.75106           3.75106               7.329
7.329
            b          0.120754          0.120754            0.208881
0.208881
            c                --                --               0.254469
0.254469
            d                --                 1                  --
1
```

Parameter Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	-3.90323	-3.99572	-3.90323	-3.85997
rho	2.98476	3.04094	2.98476	2.95686
a	6.9219	6.82651	6.9219	6.84835
b	0.118982	0.121699	0.118982	0.255061
c	--	--	0	0.408798
d	--	1.08466	--	1.34213

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0	10	6.98	2.29
1.361	10	6.36	2.452
2.451	9	4.39	1.308
3.761	9	4.63	1.564
5.258	10	3.73	0.941

Estimated Values of Interest

Model	Dose	Est Mean	Est Std	Scaled Residual
2	0	6.922	2.549	0.07208
	1.361	5.887	2.002	0.7471
	2.451	5.171	1.649	-1.42
	3.761	4.425	1.307	0.4715
	5.258	3.703	1.002	0.08592
3	0	6.827	2.516	0.1929
	1.361	5.921	2.027	0.6844
	2.451	5.215	1.671	-1.482
	3.761	4.448	1.312	0.417
	5.258	3.686	0.9859	0.1398
4	0	6.922	2.549	0.07208
	1.361	5.887	2.002	0.7471
	2.451	5.171	1.649	-1.42
	3.761	4.425	1.307	0.4715
	5.258	3.703	1.002	0.08592
5	0	6.848	2.496	0.1668
	1.361	5.979	2.042	0.59
	2.451	5.177	1.65	-1.431
	3.761	4.372	1.285	0.6022
	5.258	3.719	1.012	0.03536

Other models for which likelihoods are calculated:

Model A1: $Y_{ij} = \mu_i + e_{ij}$
$\text{Var}\{e_{ij}\} = \sigma^2$

Model A2: $Y_{ij} = \mu_i + e_{ij}$
$\text{Var}\{e_{ij}\} = \sigma_i^2$

```
  Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

  Model  R:        Yij = Mu + e(i)
           Var{e(ij)} = Sigma^2
```

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-50.12088	6	112.2418
A2	-44.44769	10	108.8954
A3	-44.70446	7	103.4089
R	-60.31932	2	124.6386
2	-47.2319	4	102.4638
3	-47.21004	5	104.4201
4	-47.2319	4	102.4638
5	-47.16859	6	106.3372

Additive constant for all log-likelihoods = -44.11. This constant added to the above values gives the log-likelihood including the term that does not depend on the model parameters.

Explanation of Tests

Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A2 vs. A1)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does Model 2 fit the data? (A3 vs. 2)

Test 5a: Does Model 3 fit the data? (A3 vs 3)
Test 5b: Is Model 3 better than Model 2? (3 vs. 2)

Test 6a: Does Model 4 fit the data? (A3 vs 4)
Test 6b: Is Model 4 better than Model 2? (4 vs. 2)

Test 7a: Does Model 5 fit the data? (A3 vs 5)
Test 7b: Is Model 5 better than Model 3? (5 vs. 3)
Test 7c: Is Model 5 better than Model 4? (5 vs. 4)

Tests of Interest

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	31.74	8	0.0001035
Test 2	11.35	4	0.02294
Test 3	0.5135	3	0.9159
Test 4	5.055	3	0.1678
Test 5a	5.011	2	0.08163
Test 5b	0.04371	1	0.8344
Test 6a	5.055	3	0.1678
Test 6b	1.421e-014	0	N/A
Test 7a	4.928	1	0.02642
Test 7b	0.0829	1	0.7734
Test 7c	0.1266	2	0.9387

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose

levels, it seems appropriate to model the data.

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate.

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here.

The p-value for Test 4 is greater than .1. Model 2 seems to adequately describe the data.

The p-value for Test 5a is less than .1. Model 3 may not adequately describe the data; you may want to consider another model.

The p-value for Test 5b is greater than .05. Model 3 does not seem to fit the data better than Model 2.

The p-value for Test 6a is greater than .1. Model 4 seems to adequately describe the data.

Degrees of freedom for Test 6b are less than or equal to 0. The Chi-Square test for fit is not valid.

The p-value for Test 7a is less than .1. Model 5 may not adequately describe the data; you may want to consider another model.

The p-value for Test 7b is greater than .05. Model 5 does not seem to fit the data better than Model 3.

The p-value for Test 7c is greater than .05. Model 5 does not seem to fit the data better than Model 4.

Benchmark Dose Computations:

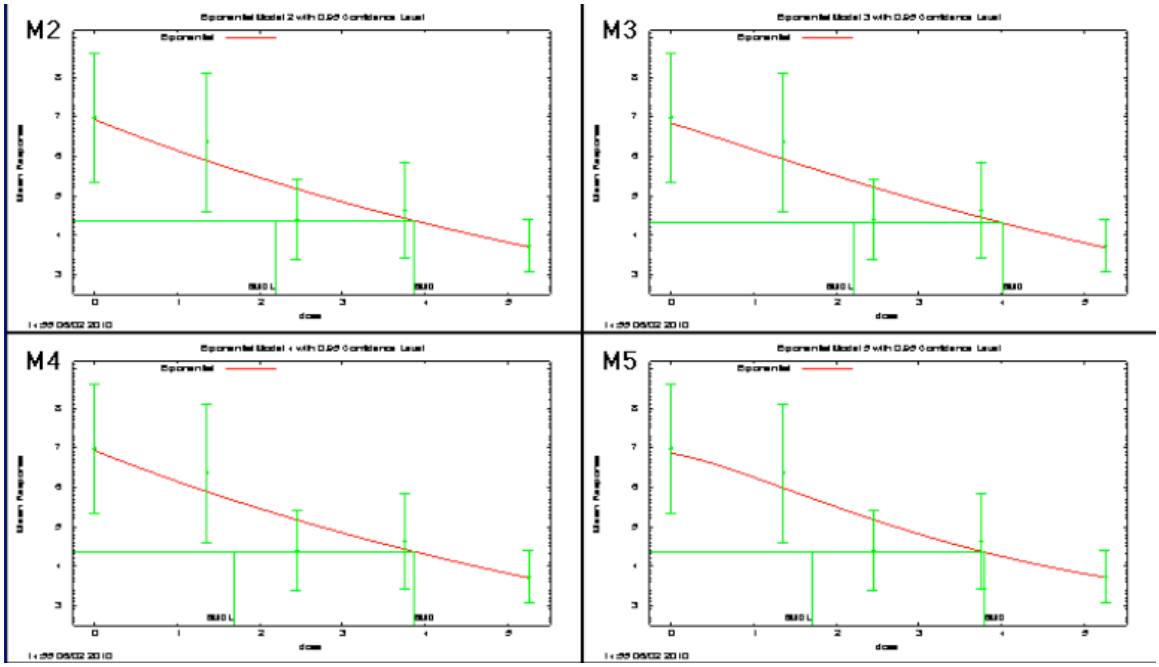
Specified Effect = 1.000000

Risk Type = Estimated standard deviations from control

Confidence Level = 0.950000

BMD and BMDL by Model

Model	BMD	BMDL
2	3.85985	2.19274
3	4.01408	2.20163
4	3.85985	1.68317
5	3.79761	1.70124


M2
M3
M4
M5

Lymphocytes, Log Dose, Historical HLS SD - Linear

```
 ================================================================
        Polynomial Model. (Version: 2.13;  Date: 04/08/2008)
        Input Data File: C:\USEPA\BMDS21\Data\lin_HLS-Lymphocytes-logdose-
historicalHLS-SD_Setting.(d)
        Gnuplot Plotting File:  C:\USEPA\BMDS21\Data\lin_HLS-Lymphocytes-logdose-
historicalHLS-SD_Setting.plt
                                            Wed Jun 02 14:49:52 2010
 ================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...


   Dependent variable = Response
   Independent variable = Dose
   Signs of the polynomial coefficients are not restricted
   The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

   Total number of dose groups = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                         lalpha =      1.19837
                            rho =            0
                         beta_0 =      6.83072
                         beta_1 =    -0.628439


           Asymptotic Correlation Matrix of Parameter Estimates

                lalpha          rho       beta_0       beta_1

    lalpha           1        -0.99         0.16        -0.19

       rho       -0.99            1        -0.16         0.19

    beta_0        0.16        -0.16            1        -0.91

    beta_1       -0.19         0.19        -0.91            1


                                Parameter Estimates

                                                    95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
         lalpha          -4.2279          1.68468            -7.52982           -
0.925975
            rho          3.18622          1.02458             1.17808
```

5.19435
beta_0 6.68827 0.512056 5.68466
7.69189
beta_1 -0.574725 0.123487 -0.816754 -
0.332695

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	10	6.98	6.69	2.29	2.49	0.37
1.361	10	6.36	5.91	2.45	2.04	0.702
2.451	9	4.39	5.28	1.31	1.71	-1.56
3.761	9	4.63	4.53	1.56	1.34	0.232
5.258	10	3.73	3.67	0.941	0.957	0.21

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
Model A3 uses any fixed variance parameters that
were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-50.120881	6	112.241762
A2	-44.447695	10	108.895390
A3	-44.704461	7	103.408922
fitted	-47.302522	4	102.605045
R	-60.319315	2	124.638631

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	31.7432	8	0.0001035

Test 2	11.3464	4	0.02294
Test 3	0.513532	3	0.9159
Test 4	5.19612	3	0.158

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels It seems appropriate to model the data

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems to adequately describe the data

Benchmark Dose Computation

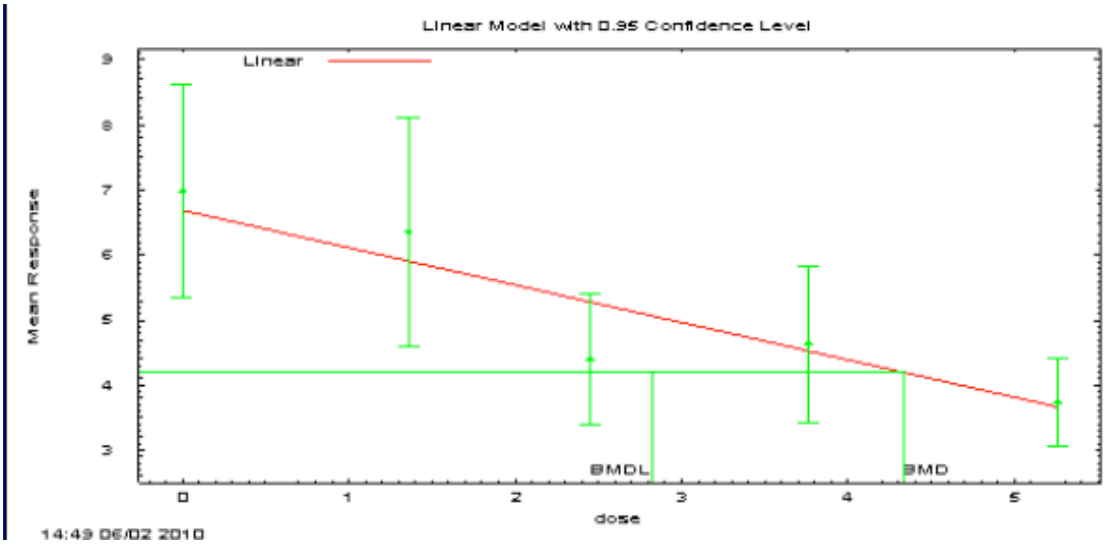
Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

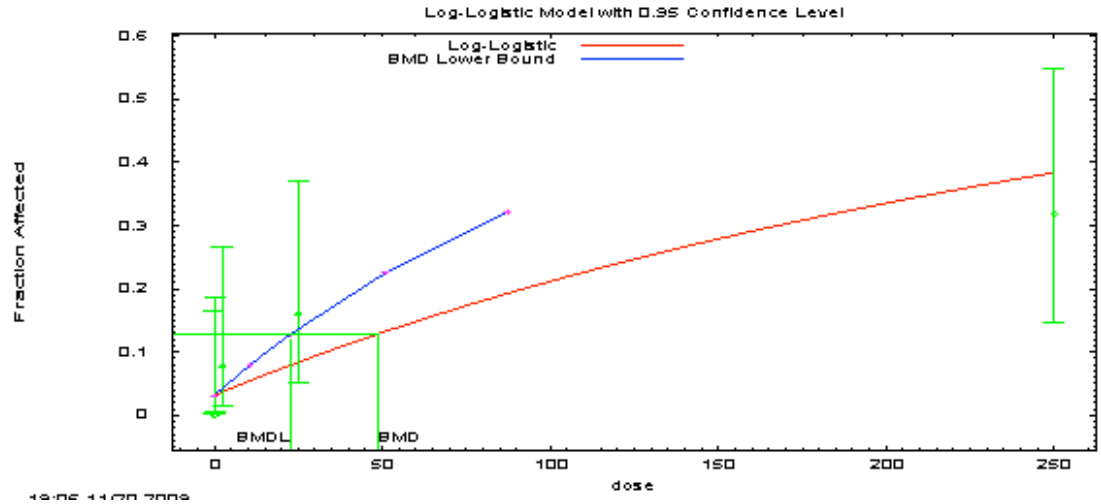
BMD = 4.33789

BMDL = 2.82726



Appendix D: Benchmark Dose Modeling of Zhu et al. (1987)

Study: Zhu et al. (1987)
Endpoint: fatty liver




```
==========================================================================

        Logistic Model. (Version: 2.12; Date: 05/16/2008)

        Input Data File: C:\USEPA\BMDS21\Data\lnlZhu-liverSetting.(d)

        Gnuplot Plotting File:  C:\USEPA\BMDS21\Data\lnlZhu-liverSetting.plt

                                                Fri Nov 20 19:06:36 2009
 ========================================================================


 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]


   Dependent variable = Response
   Independent variable = Dose
   Slope parameter is restricted as slope >= 1

   Total number of observations = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



   User has chosen the log transformed model
```

Default Initial Parameter Values
background = 0
intercept = -5.81209
slope = 1

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -slope
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	background	intercept
background	1	-0.47
intercept	-0.47	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
background	0.0314502	*	*	*
intercept	-6.07894	*	*	*
slope	1	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-31.8035	5			
Fitted model	-34.7347	2	5.86251	3	0.1185
Reduced model	-41.162	1	18.717	4	0.0008932

AIC: 73.4695

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0315	0.786	0.000	25	-0.901
0.2500	0.0320	0.704	0.000	22	-0.853
2.5000	0.0370	0.961	2.000	26	1.080
25.0000	0.0839	2.098	4.000	25	1.372
250.0000	0.3841	8.451	7.000	22	-0.636

Chi^2 = 4.99 d.f. = 3 P-value = 0.1723

Benchmark Dose Computation

Specified effect = 0.1

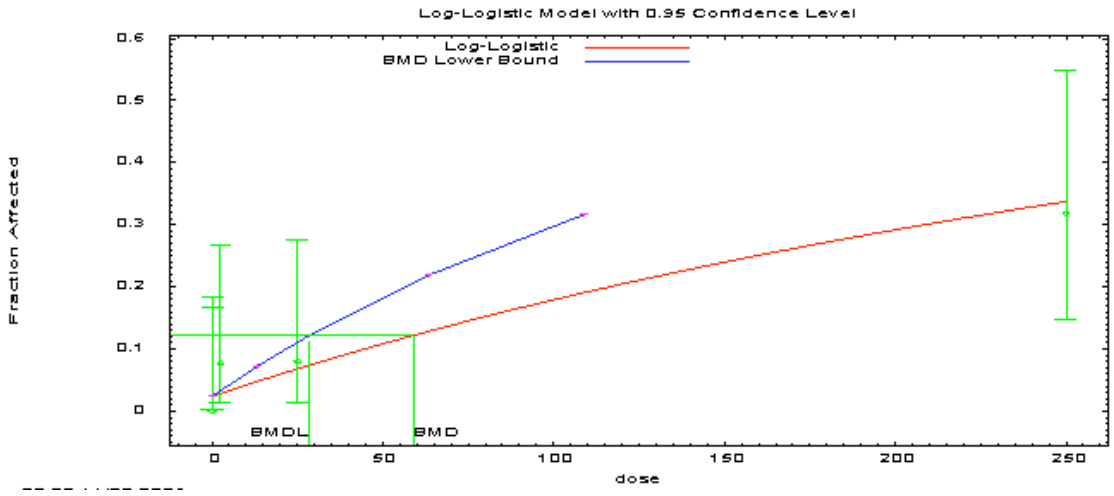
Risk Type = Extra risk

```
Confidence level =           0.95

             BMD =        48.5074

            BMDL =        22.6332
```

Study: Zhu et al. (1987)
Endpoint: spleen




```
==========================================================================

        Logistic Model. (Version: 2.12; Date: 05/16/2008)

        Input Data File: C:\USEPA\BMDS21\Data\lnlZhu-spleen-6moSetting.(d)

        Gnuplot Plotting File:  C:\USEPA\BMDS21\Data\lnlZhu-spleen-6moSetting.plt

                                                Fri Nov 20 20:27:35 2009
==========================================================================


 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]


   Dependent variable = Response
   Independent variable = Dose
   Slope parameter is restricted as slope >= 1

   Total number of observations = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



   User has chosen the log transformed model


                 Default Initial Parameter Values
                    background =            0
                     intercept =     -6.10214
                         slope =            1
```

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -slope
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	background	intercept
background	1	-0.36
intercept	-0.36	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
background	0.024055	*	*	*
intercept	-6.27229	*	*	*
slope	1	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-27.781	5			
Fitted model	-29.7325	2	3.90306	3	0.2721
Reduced model	-36.7652	1	17.9685	4	0.001252

AIC: 63.465

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0241	0.601	0.000	25	-0.785
0.2500	0.0245	0.539	0.000	22	-0.744
2.5000	0.0286	0.745	2.000	26	1.476
25.0000	0.0680	1.701	2.000	25	0.237
250.0000	0.3370	7.414	7.000	22	-0.187

Chi^2 = 3.44 d.f. = 3 P-value = 0.3287

Benchmark Dose Computation

Specified effect = 0.1

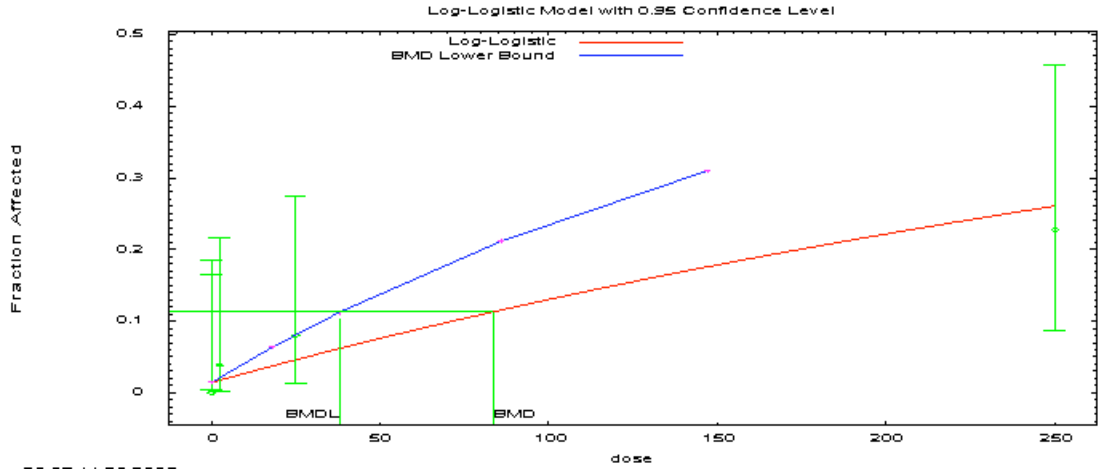
Risk Type = Extra risk

Confidence level = 0.95

BMD = 58.8542

BMDL = 28.2609

Study: Zhu et al. (1987)
Endpoint: heavy fatty liver degeneration




```
=================================================================================

        Logistic Model. (Version: 2.12; Date: 05/16/2008)

        Input Data File: C:\USEPA\BMDS21\Data\lnlZhu-fattydegenerationSetting.(d)

        Gnuplot Plotting File:  C:\USEPA\BMDS21\Data\lnlZhu-
fattydegenerationSetting.plt
                                                  Fri Nov 20 20:37:54 2009
 ================================================================================


 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~


   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]


   Dependent variable = Response
   Independent variable = Dose
   Slope parameter is restricted as slope >= 1

   Total number of observations = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



   User has chosen the log transformed model


                 Default Initial Parameter Values
                    background =            0
                     intercept =     -6.47908
                         slope =            1


          Asymptotic Correlation Matrix of Parameter Estimates
```

(*** The model parameter(s) -slope
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	background	intercept
background	1	-0.4
intercept	-0.4	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
background	0.0142098	*	*	*
intercept	-6.623	*	*	*
slope	1	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-22.999	5			
Fitted model	-24.2652	2	2.53239	3	0.4695
Reduced model	-29.3916	1	12.7853	4	0.01237

AIC: 52.5303

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0142	0.355	0.000	25	-0.600
0.2500	0.0145	0.320	0.000	22	-0.570
2.5000	0.0175	0.454	1.000	26	0.817
25.0000	0.0459	1.148	2.000	25	0.814
250.0000	0.2601	5.723	5.000	22	-0.351

Chi^2 = 2.14 d.f. = 3 P-value = 0.5443

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 83.5773

BMDL = 38.1362

Appendix E: Benchmark Dose Modeling of OECD (2004)

OECD-Pups Alive on Day 4 1SD – Exponential

```
 ================================================================
        Exponential Model. (Version: 1.61;  Date: 7/24/2009)
        Input Data File: C:\USEPA\BMDS21\Data\exp_OECD-birth-pupsday4_Setting.(d)
        Gnuplot Plotting File:
                                              Sun Jun 13 22:11:06 2010
 ================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function by Model:
      Model 2:     Y[dose] = a * exp{sign * b * dose}
      Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
      Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
      Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

    Note: Y[dose] is the median response for exposure = dose;
          sign = +1 for increasing trend in data;
          sign = -1 for decreasing trend.

      Model 2 is nested within Models 3 and 4.
      Model 3 is nested within Model 5.
      Model 4 is nested within Model 5.


   Dependent variable = Response
   Independent variable = Dose
   Data are assumed to be distributed: normally
   Variance Model: exp(lnalpha +rho *ln(Y[dose]))
   The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

   MLE solution provided: Exact


                                Initial Parameter Values

     Variable          Model 2           Model 3           Model 4           Model 5
     --------          -------           -------           -------           -------
      lnalpha           5.99242           5.99242              5.99242
5.99242
          rho          -1.86471          -1.86471            -1.86471          -
1.86471
            a           7.22768           3.58254                15.75
15.75
            b        0.00196403       -8.246e-007           0.00185669
0.00185669
            c                --                --            0.000253968
0.000253968
```

d	--	2	--
1			

Parameter Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	12.6863	5.58675	12.6863	5.59885
rho	-4.3033	-1.7118	-4.3033	-1.71698
a	15.3575	14.902	15.3575	14.8741
b	0.000902708	0.00163543	0.000902708	0.00424764
c	--	--	0	0.256581
d	--	2.30684	--	13.9314

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0	11	14.8	1.8
60	12	15	1.9
200	10	13.7	1.3
700	9	4	5.6

Estimated Values of Interest

Model	Dose	Est Mean	Est Std	Scaled Residual
2	0	15.36	1.593	-1.161
	60	14.55	1.79	0.875
	200	12.82	2.349	1.184
	700	8.164	6.205	-2.013
3	0	14.9	1.618	-0.2091
	60	14.83	1.625	0.3587
	200	13.81	1.727	-0.2059
	700	3.802	5.209	0.1143
4	0	15.36	1.593	-1.161
	60	14.55	1.79	0.875
	200	12.82	2.349	1.184
	700	8.164	6.205	-2.013
5	0	14.87	1.619	-0.1517
	60	14.87	1.619	0.2695
	200	13.79	1.728	-0.1659
	700	3.816	5.205	0.1058

Other models for which likelihoods are calculated:

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

```
  Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

  Model  R:        Yij = Mu + e(i)
           Var{e(ij)} = Sigma^2
```

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-64.80532	5	139.6106
A2	-51.19334	8	118.3867
A3	-52.36184	6	116.7237
R	-90.21303	2	184.4261
2	-58.07757	4	124.1551
3	-52.43031	5	114.8606
4	-58.07757	4	124.1551
5	-52.39627	6	116.7925

Additive constant for all log-likelihoods = -38.6. This constant added to the above values gives the log-likelihood including the term that does not depend on the model parameters.

Explanation of Tests

Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A2 vs. A1)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does Model 2 fit the data? (A3 vs. 2)

Test 5a: Does Model 3 fit the data? (A3 vs 3)
Test 5b: Is Model 3 better than Model 2? (3 vs. 2)

Test 6a: Does Model 4 fit the data? (A3 vs 4)
Test 6b: Is Model 4 better than Model 2? (4 vs. 2)

Test 7a: Does Model 5 fit the data? (A3 vs 5)
Test 7b: Is Model 5 better than Model 3? (5 vs. 3)
Test 7c: Is Model 5 better than Model 4? (5 vs. 4)

Tests of Interest

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	78.04	6	< 0.0001
Test 2	27.22	3	< 0.0001
Test 3	2.337	2	0.3108
Test 4	11.43	2	0.003294
Test 5a	0.1369	1	0.7113
Test 5b	11.29	1	0.0007774
Test 6a	11.43	2	0.003294
Test 6b	-2.842e-014	0	N/A
Test 7a	0.06887	0	N/A
Test 7b	0.06807	1	0.7942
Test 7c	11.36	2	0.003409

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose

levels, it seems appropriate to model the data.

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate.

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here.

The p-value for Test 4 is less than .1. Model 2 may not adequately describe the data; you may want to consider another model.

The p-value for Test 5a is greater than .1. Model 3 seems to adequately describe the data.

The p-value for Test 5b is less than .05. Model 3 appears to fit the data better than Model 2.

The p-value for Test 6a is less than .1. Model 4 may not adequately describe the data; you may want to consider another model.

Degrees of freedom for Test 6b are less than or equal to 0. The Chi-Square test for fit is not valid.

Degrees of freedom for Test 7a are less than or equal to 0. The Chi-Square test for fit is not valid.

The p-value for Test 7b is greater than .05. Model 5 does not seem to fit the data better than Model 3.

The p-value for Test 7c is less than .05. Model 5 appears to fit the data better than Model 4.

Benchmark Dose Computations:

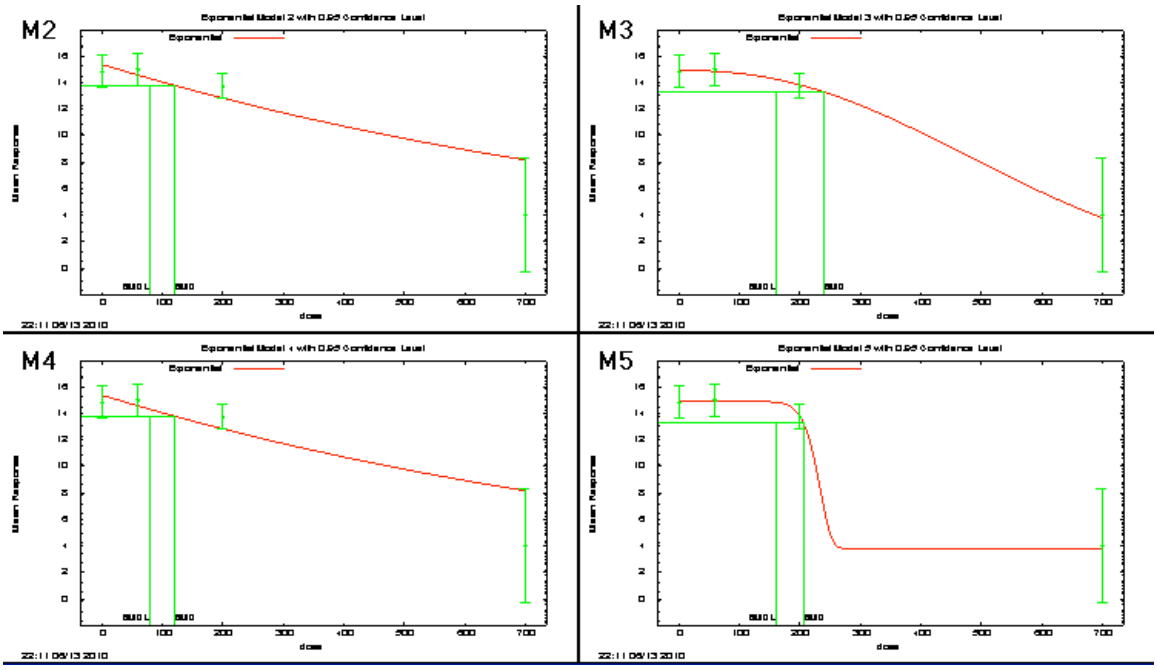
Specified Effect = 1.000000

Risk Type = Estimated standard deviations from control

Confidence Level = 0.950000

BMD and BMDL by Model

Model	BMD	BMDL
2	121.32	80.1334
3	239.373	161.176
4	121.32	80.1334
5	206.25	162.02


M2
M3
M4
M5

```
 ================================================================
        Polynomial Model. (Version: 2.13;  Date: 04/08/2008)
        Input Data File: C:\USEPA\BMDS21\Data\ply_OECD-birth-pupsday4_Setting.(d)
        Gnuplot Plotting File:  C:\USEPA\BMDS21\Data\ply_OECD-birth-
pupsday4_Setting.plt
                                          Sun Jun 13 22:16:59 2010
 ================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...


   Dependent variable = Response
   Independent variable = Dose
   Signs of the polynomial coefficients are not restricted
   The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                 Default Initial Parameter Values
                        lalpha =      2.18605
                           rho =            0
                        beta_0 =      14.9492
                        beta_1 =  -0.00185795
                        beta_2 = -1.97026e-005


          Asymptotic Correlation Matrix of Parameter Estimates

               lalpha          rho       beta_0       beta_1       beta_2

    lalpha          1        -0.97       -0.018         0.05       -0.097

       rho        -0.97            1       0.0075       -0.034        0.082

    beta_0       -0.018       0.0075            1         -0.7         0.52

    beta_1         0.05       -0.034         -0.7            1        -0.89

    beta_2       -0.097        0.082         0.52        -0.89            1


                              Parameter Estimates

                                                      95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
```

```
Limit
         lalpha          5.57656          0.96112             3.69279
7.46032
           rho         -1.70701         0.391337            -2.47402           -
0.940008
         beta_0          14.9057         0.419226              14.084
15.7273
         beta_1     -0.000827206       0.00503835          -0.0107022
0.00904777
         beta_2    -2.15132e-005     8.35669e-006       -3.78921e-005       -5.13443e-
006
```

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	11	14.8	14.9	1.8	1.62	-0.216
60	12	15	14.8	1.9	1.63	0.47
200	10	13.7	13.9	1.3	1.72	-0.33
700	9	4	3.79	5.6	5.22	0.124

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
Model A3 uses any fixed variance parameters that
were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-64.805324	5	139.610648
A2	-51.193341	8	118.386683
A3	-52.361840	6	116.723679
fitted	-52.482747	5	114.965494
R	-90.213033	2	184.426065

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	78.0394	6	<.0001
Test 2	27.224	3	<.0001
Test 3	2.337	2	0.3108
Test 4	0.241815	1	0.6229

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels It seems appropriate to model the data

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems to adequately describe the data

Benchmark Dose Computation

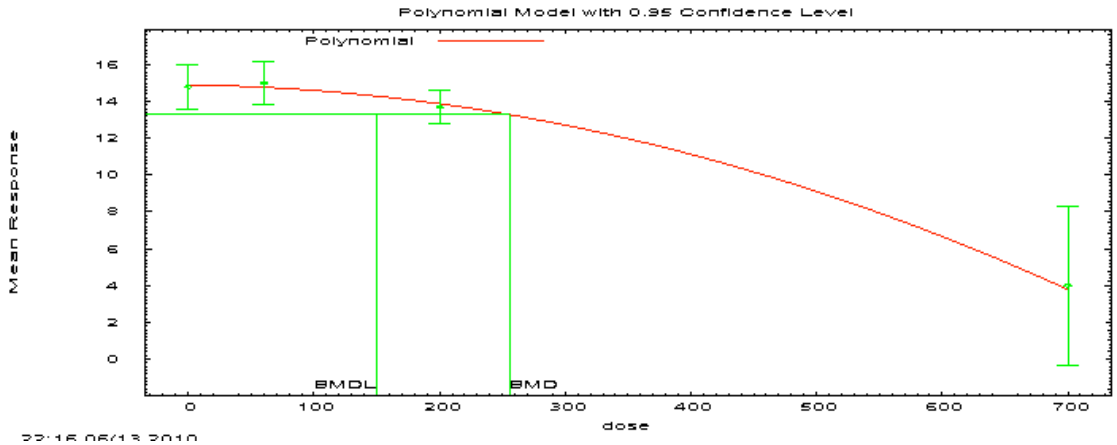
Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 255.846

BMDL = 149.421



```
 ================================================================
        Power Model. (Version: 2.15;  Date: 04/07/2008)
        Input Data File: C:\USEPA\BMDS21\Data\pow_OECD-birth-pupsday4_Setting.(d)
        Gnuplot Plotting File:  C:\USEPA\BMDS21\Data\pow_OECD-birth-
pupsday4_Setting.plt
                                          Sun Jun 13 22:19:13 2010
 ================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = control + slope * dose^power


   Dependent variable = Response
   Independent variable = Dose
   The power is restricted to be greater than or equal to 1
   The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                         lalpha =      2.18605
                            rho =            0
                        control =            4
                          slope =       16.871
                          power =    -0.104462


           Asymptotic Correlation Matrix of Parameter Estimates

                lalpha          rho      control        slope        power

    lalpha           1        -0.99       -0.047       -0.089        -0.13

       rho       -0.99            1        0.061        0.086         0.13

   control      -0.047        0.061            1        -0.61         -0.6

     slope      -0.089        0.086        -0.61            1            1

     power       -0.13         0.13         -0.6            1            1


                                Parameter Estimates

                                                      95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
```

```
Limit
         lalpha          5.59406          1.76048             2.14359
9.04454
           rho         -1.71391         0.673367            -3.03369           -
0.394138
       control          14.9169         0.374541             14.1828
15.651
         slope    -5.74038e-005      0.000181674        -0.000413479
0.000298671
         power          1.85817         0.483752            0.910033
2.80631
```

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	11	14.8	14.9	1.8	1.62	-0.24
60	12	15	14.8	1.9	1.63	0.423
200	10	13.7	13.8	1.3	1.73	-0.245
700	9	4	3.81	5.6	5.21	0.11

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
Model A3 uses any fixed variance parameters that
were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-64.805324	5	139.610648
A2	-51.193341	8	118.386683
A3	-52.361840	6	116.723679
fitted	-52.460561	5	114.921123
R	-90.213033	2	184.426065

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	78.0394	6	<.0001
Test 2	27.224	3	<.0001
Test 3	2.337	2	0.3108
Test 4	0.197444	1	0.6568

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels It seems appropriate to model the data

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems to adequately describe the data

Benchmark Dose Computation

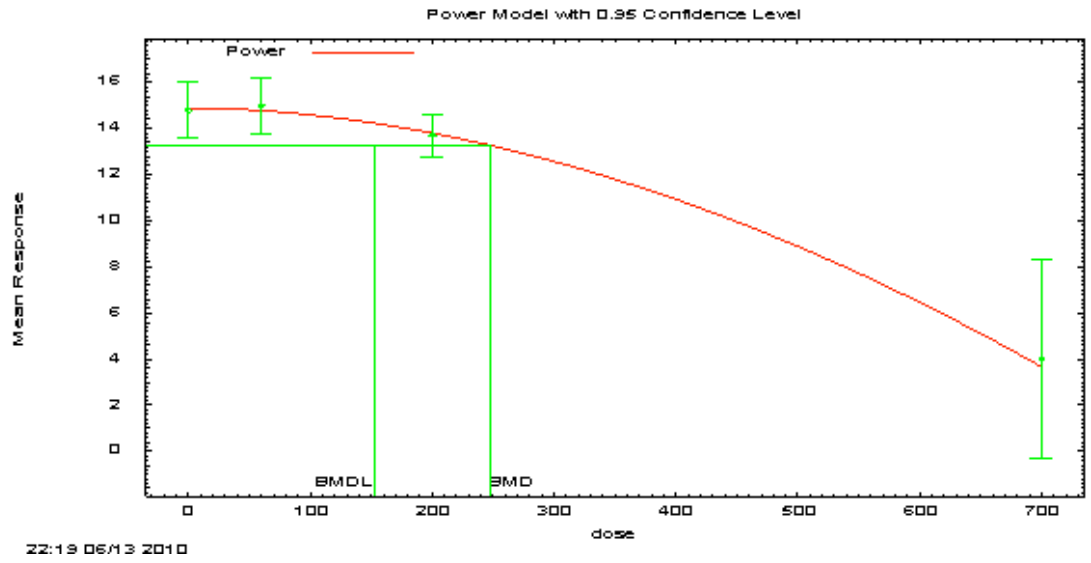
Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 248.216

BMDL = 153.084



```
 ================================================================
        Exponential Model. (Version: 1.61;  Date: 7/24/2009)
        Input Data File: C:\USEPA\BMDS21\Data\exp_OECD-birth-index_Setting.(d)
        Gnuplot Plotting File:
                                              Wed Jul 28 11:00:00 2010
 ================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function by Model:
      Model 2:     Y[dose] = a * exp{sign * b * dose}
      Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
      Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
      Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

    Note: Y[dose] is the median response for exposure = dose;
          sign = +1 for increasing trend in data;
          sign = -1 for decreasing trend.

      Model 2 is nested within Models 3 and 4.
      Model 3 is nested within Model 5.
      Model 4 is nested within Model 5.


   Dependent variable = Response
   Independent variable = Dose
   Data are assumed to be distributed: normally
   Variance Model: exp(lnalpha +rho *ln(Y[dose]))
   The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

   MLE solution provided: Exact


                                Initial Parameter Values

     Variable          Model 2           Model 3           Model 4           Model 5
     --------          -------           -------           -------           -------
      lnalpha           52.9161           52.9161              52.9161
52.9161
          rho          -10.8897          -10.8897             -10.8897          -
10.8897
            a             80.128            80.128              101.115
101.115
            b       0.000438051       0.000438051          0.000864797
0.000864797
            c                --                --               0.354052
0.354052
            d                --                 1                    --
1
```

Parameter Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	57.8453	46.0602	57.8453	47.0807
rho	-11.9536	-9.38104	-11.9536	-9.60754
a	97.0573	96.135	97.0573	95.846
b	0.000371598	0.000708097	0.000371598	0.00429424
c	--	--	0	0.721664
d	--	1.5534	--	11.129

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0	11	96.3	6.5
60	12	95.8	4.8
200	10	90.5	5.1
700	10	71.6	26.2

Estimated Values of Interest

Model	Dose	Est Mean	Est Std	Scaled Residual
2	0	97.06	4.84	-0.5189
	60	94.92	5.53	0.5529
	200	90.11	7.547	0.1653
	700	74.83	22.91	-0.4454
3	0	96.13	5.025	0.1089
	60	95.43	5.202	0.2488
	200	91.63	6.294	-0.5669
	700	68.69	24.31	0.3783
4	0	97.06	4.84	-0.5189
	60	94.92	5.53	0.5529
	200	90.11	7.547	0.1653
	700	74.83	22.91	-0.4454
5	0	95.85	5.063	0.2974
	60	95.85	5.063	-0.03149
	200	91.37	6.373	-0.4292
	700	69.17	24.26	0.3169

Other models for which likelihoods are calculated:

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

Model R: Yij = Mu + e(i)
Var{e(ij)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-131.2566	5	272.5131
A2	-107.7633	8	231.5267
A3	-109.2007	6	230.4013
R	-141.2441	2	286.4883
2	-110.8975	4	229.795
3	-109.3519	5	228.7037
4	-110.8975	4	229.795
5	-109.2154	6	230.4307

Additive constant for all log-likelihoods = -39.51. This constant added to the above values gives the log-likelihood including the term that does not depend on the model parameters.

Explanation of Tests

Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A2 vs. A1)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does Model 2 fit the data? (A3 vs. 2)

Test 5a: Does Model 3 fit the data? (A3 vs 3)
Test 5b: Is Model 3 better than Model 2? (3 vs. 2)

Test 6a: Does Model 4 fit the data? (A3 vs 4)
Test 6b: Is Model 4 better than Model 2? (4 vs. 2)

Test 7a: Does Model 5 fit the data? (A3 vs 5)
Test 7b: Is Model 5 better than Model 3? (5 vs. 3)
Test 7c: Is Model 5 better than Model 4? (5 vs. 4)

Tests of Interest

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	66.96	6	< 0.0001
Test 2	46.99	3	< 0.0001
Test 3	2.875	2	0.2376
Test 4	3.394	2	0.1833
Test 5a	0.3024	1	0.5824
Test 5b	3.091	1	0.07871
Test 6a	3.394	2	0.1833
Test 6b	-1.705e-013	0	N/A
Test 7a	0.0294	0	N/A
Test 7b	0.273	1	0.6013
Test 7c	3.364	2	0.186

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels, it seems appropriate to model the data.

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate.

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here.

The p-value for Test 4 is greater than .1. Model 2 seems to adequately describe the data.

The p-value for Test 5a is greater than .1. Model 3 seems to adequately describe the data.

The p-value for Test 5b is greater than .05. Model 3 does not seem to fit the data better than Model 2.

The p-value for Test 6a is greater than .1. Model 4 seems to adequately describe the data.

Degrees of freedom for Test 6b are less than or equal to 0. The Chi-Square test for fit is not valid.

Degrees of freedom for Test 7a are less than or equal to 0. The Chi-Square test for fit is not valid.

The p-value for Test 7b is greater than .05. Model 5 does not seem to fit the data better than Model 3.

The p-value for Test 7c is greater than .05. Model 5 does not seem to fit the data better than Model 4.

Benchmark Dose Computations:

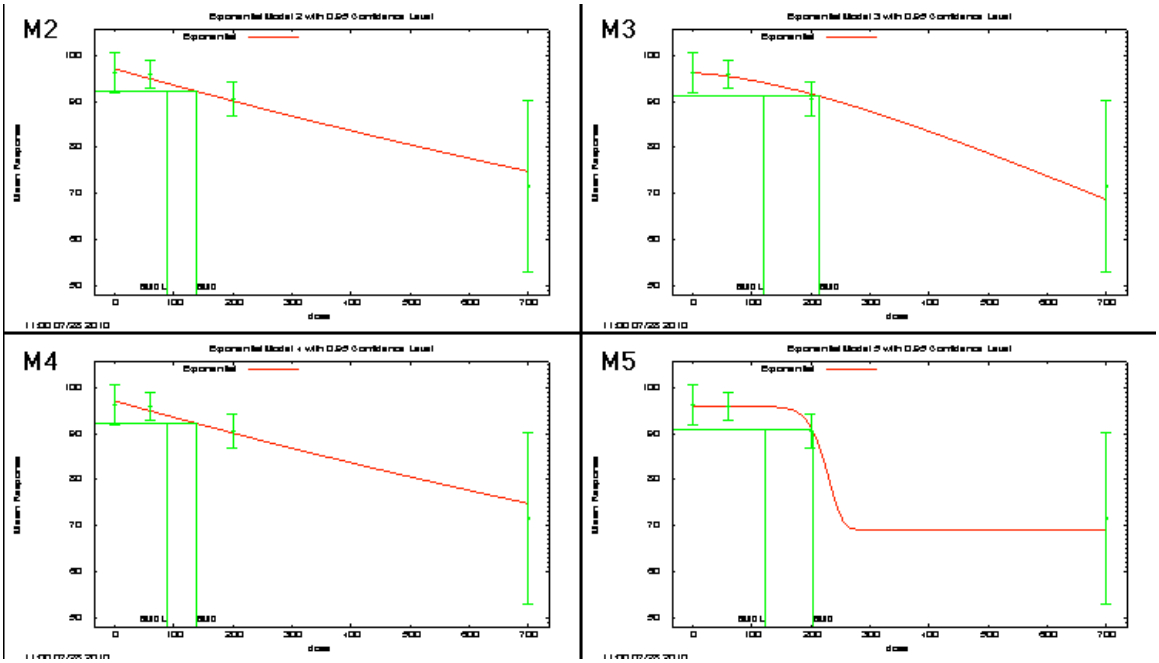
Specified Effect = 1.000000

Risk Type = Estimated standard deviations from control

Confidence Level = 0.950000

BMD and BMDL by Model

Model	BMD	BMDL
2	137.667	88.4787
3	214.899	119.71
4	137.667	88.4445
5	202.441	123.705


M2
M3
M4
M5
Exponential
Mean Response
dose

```
 ================================================================
        Polynomial Model. (Version: 2.13;  Date: 04/08/2008)
        Input Data File: C:\USEPA\BMDS21\Data\ply_OECD-birth-index_Setting.(d)
        Gnuplot Plotting File:  C:\USEPA\BMDS21\Data\ply_OECD-birth-index_Setting.plt
                                          Wed Jul 28 11:01:19 2010
 ================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...


   Dependent variable = Response
   Independent variable = Dose
   Signs of the polynomial coefficients are not restricted
   The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                         lalpha =       5.2026
                            rho =            0
                         beta_0 =      96.7661
                         beta_1 =   -0.0275145
                         beta_2 = -1.20887e-005


           Asymptotic Correlation Matrix of Parameter Estimates

                lalpha          rho       beta_0       beta_1       beta_2

    lalpha           1           -1       -0.021         0.15        -0.32

       rho          -1            1        0.015        -0.14         0.32

    beta_0       -0.021        0.015            1        -0.69         0.46

    beta_1         0.15        -0.14        -0.69            1        -0.84

    beta_2        -0.32         0.32         0.46        -0.84            1


                                Parameter Estimates

                                                        95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
         lalpha          45.9172           7.4789             31.2588
60.5755
```

```
           rho         -9.34782          1.66687           -12.6148           -
6.08083
        beta_0          96.2374           1.3683            93.5556
98.9193
        beta_1       -0.0152503        0.0184832         -0.0514768
0.0209762
        beta_2    -3.44801e-005     3.83981e-005        -0.000109739        4.07788e-
005
```

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	11	96.3	96.2	6.5	5.02	0.0413
60	12	95.8	95.2	4.8	5.28	0.395
200	10	90.5	91.8	5.1	6.26	-0.661
700	10	71.6	68.7	26.2	24.3	0.381

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
Model A3 uses any fixed variance parameters that
were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-131.256575	5	272.513150
A2	-107.763335	8	231.526670
A3	-109.200666	6	230.401333
fitted	-109.476033	5	228.952065
R	-141.244134	2	286.488268

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value

Test 1	66.9616	6	<.0001
Test 2	46.9865	3	<.0001
Test 3	2.87466	2	0.2376
Test 4	0.550732	1	0.458

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems to adequately describe the data

Benchmark Dose Computation

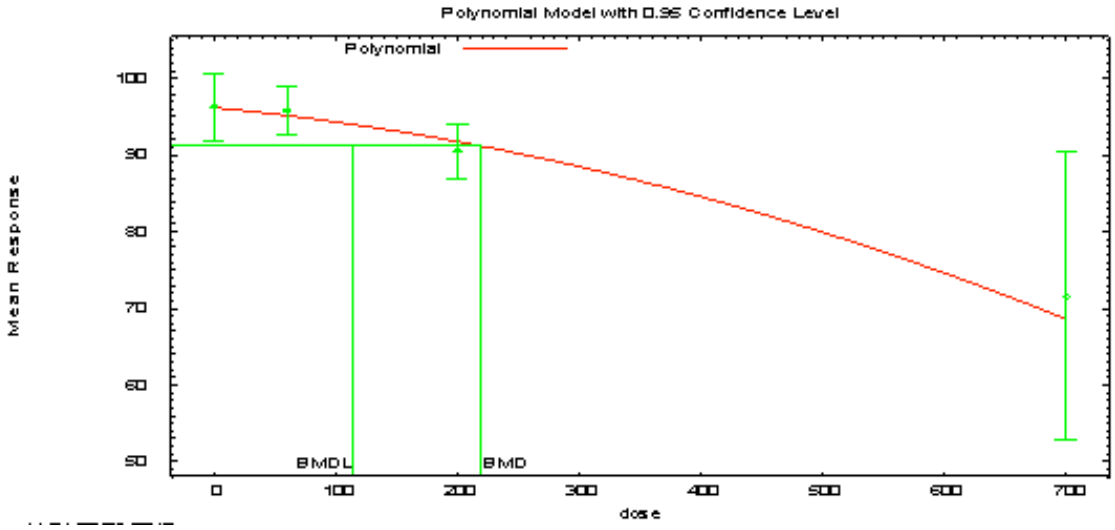
Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 219.912

BMDL = 114.151



```
 ================================================================
        Power Model. (Version: 2.15;  Date: 04/07/2008)
        Input Data File: C:\USEPA\BMDS21\Data\pow_OECD-birth-index_Setting.(d)
        Gnuplot Plotting File:  C:\USEPA\BMDS21\Data\pow_OECD-birth-index_Setting.plt
                                          Wed Jul 28 11:02:43 2010
 ================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = control + slope * dose^power


   Dependent variable = Response
   Independent variable = Dose
   The power is restricted to be greater than or equal to 1
   The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                         lalpha =       5.2026
                            rho =            0
                        control =         96.3
                          slope =      -94.805
                          power =    -0.205313


           Asymptotic Correlation Matrix of Parameter Estimates

                lalpha          rho      control        slope        power

    lalpha           1           -1        -0.16        -0.11        -0.21

       rho          -1            1         0.16         0.11         0.21

   control       -0.16         0.16            1        -0.59        -0.55

     slope       -0.11         0.11        -0.59            1         0.99

     power       -0.21         0.21        -0.55         0.99            1


                                Parameter Estimates

                                                      95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
```

lalpha	46.0619	14.2684	18.0963	74.0275
rho	-9.38101	3.14401	-15.5432	-3.21886
control	96.1549	1.24079	93.723	98.5868
slope	-0.00208381	0.00496587	-0.0118167	0.00764912
power	1.44801	0.369428	0.723946	2.17208

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	11	96.3	96.2	6.5	5.02	0.0958
60	12	95.8	95.4	4.8	5.22	0.284
200	10	90.5	91.7	5.1	6.28	-0.594
700	10	71.6	68.7	26.2	24.3	0.377

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
Model A3 uses any fixed variance parameters that
were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-131.256575	5	272.513150
A2	-107.763335	8	231.526670
A3	-109.200666	6	230.401333
fitted	-109.377751	5	228.755503
R	-141.244134	2	286.488268

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	66.9616	6	<.0001
Test 2	46.9865	3	<.0001
Test 3	2.87466	2	0.2376
Test 4	0.35417	1	0.5518

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels It seems appropriate to model the data

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems to adequately describe the data

Benchmark Dose Computation

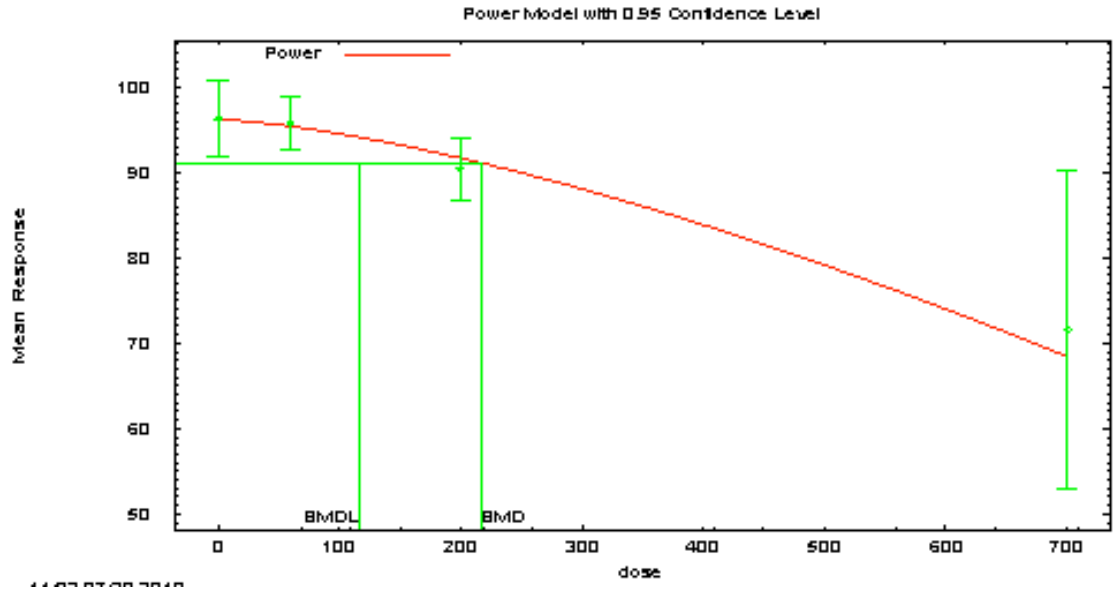
Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 216.653

BMDL = 117.36



```
 ================================================================
        Polynomial Model. (Version: 2.13;  Date: 04/08/2008)
        Input Data File: C:\USEPA\BMDS21\Data\lin_OECD-birth-index_Setting.(d)
        Gnuplot Plotting File:  C:\USEPA\BMDS21\Data\lin_OECD-birth-index_Setting.plt
                                          Wed Jul 28 11:04:17 2010
 ================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...


   Dependent variable = Response
   Independent variable = Dose
   Signs of the polynomial coefficients are not restricted
   The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                         lalpha =       5.2026
                            rho =            0
                         beta_0 =      97.2888
                         beta_1 =   -0.0364116


           Asymptotic Correlation Matrix of Parameter Estimates

                 lalpha          rho       beta_0       beta_1

    lalpha            1           -1         0.23        -0.34

       rho           -1            1        -0.23         0.34

    beta_0         0.23        -0.23            1        -0.71

    beta_1        -0.34         0.34        -0.71            1


                                Parameter Estimates

                                                      95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
         lalpha          53.5997          8.56982             36.8032
70.3962
            rho         -11.0242          1.90855            -14.7649            -
7.28352
         beta_0          97.0392          1.36686             94.3602
```

99.7182
beta_1 -0.0340974 0.014694 -0.0628971 -0.00529779

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	11	96.3	97	6.5	4.86	-0.504
60	12	95.8	95	4.8	5.47	0.511
200	10	90.5	90.2	5.1	7.26	0.122
700	10	71.6	73.2	26.2	23	-0.216

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
Model A3 uses any fixed variance parameters that
were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-131.256575	5	272.513150
A2	-107.763335	8	231.526670
A3	-109.200666	6	230.401333
fitted	-110.485188	4	228.970376
R	-141.244134	2	286.488268

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	66.9616	6	<.0001
Test 2	46.9865	3	<.0001
Test 3	2.87466	2	0.2376
Test 4	2.56904	2	0.2768

The p-value for Test 1 is less than .05. There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1. A non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is greater than .1. The modeled variance appears
 to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems
to adequately describe the data

Benchmark Dose Computation

Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 142.576

BMDL = 95.6878